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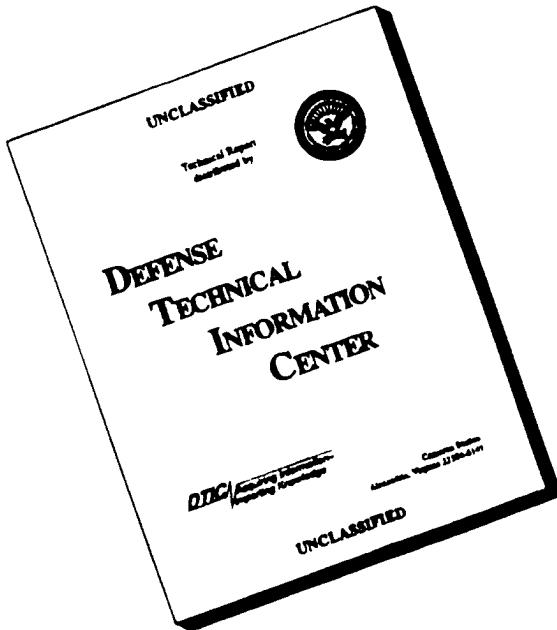
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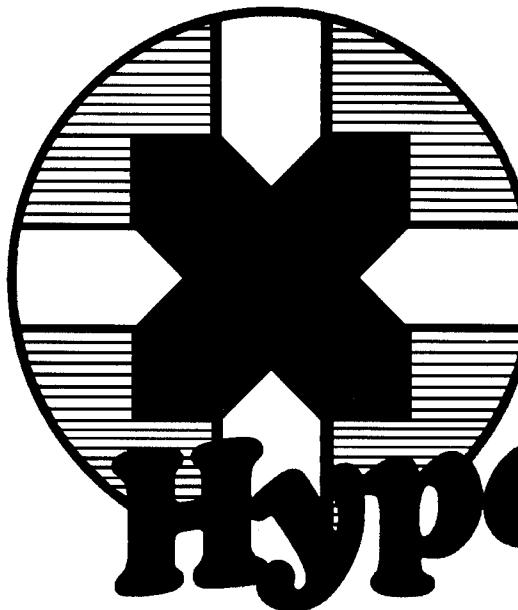
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Hypoxia

AND THE
BRAIN

JOHN R. SUTTON, M.D.

CHARLES S. HOUSTON, M.D.

— GEOFFREY COATES, M.D.

HYPOXIA AND THE BRAIN

HYPOXIA AND THE BRAIN

**PROCEEDINGS OF THE 9TH INTERNATIONAL HYPOXIA SYMPOSIUM AT LAKE
LOUISE, CANADA, FEBRUARY 14-18, 1995.**

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HYPOXIA AND THE BRAIN

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AND GEOFFREY COATES

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The Ninth International Hypoxia Symposium is considered the best yet. Its success has been due to the outstanding calibre of the Faculty and the participants and the continuing dedication of Ingrid Ellis (McMaster University), Gerald Thompson (Arctic Institute of North America) and Lyndall Burke (University of Sydney). This year we were delighted to have Ruth and Peter McHugh (McMaster University) on site to ensure that everything ran smoothly. A special word of thanks to Jacqueline Dolan who generously donated her harp and voice to maintain the unbroken tradition since Hypoxia moved from Banff to Lake Louise a decade ago.

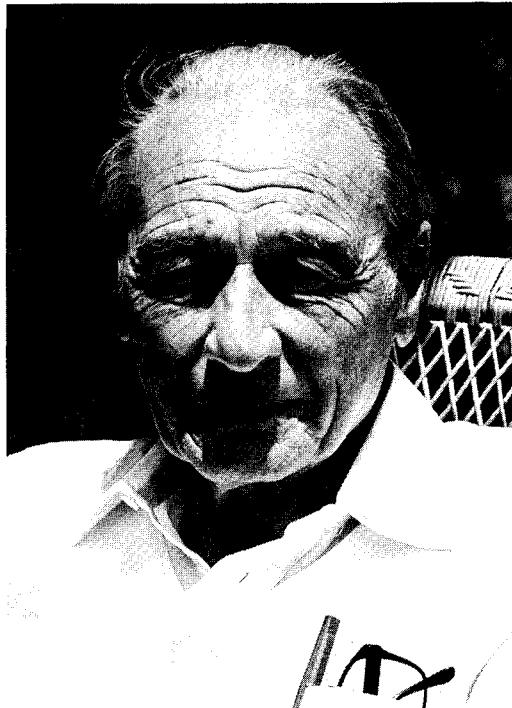
This year the exceptional co-operation of Phillip Smith, Convention Manager with Chateau Lake Louise and his staff, must also be acknowledged, they were very professional and a delight to work with.

We thank the AINA, Fisons, Faculty of Health Sciences McMaster, Merck Frosst Canada, Sandoz France and the Faculty of Health Sciences University of Sydney for their financial support.

In the final analysis, as always it has been the participants who make the biennial pilgrimage to Lake Louise, Canada who this year came in increasing numbers who have ensured its success.

Our 10th International Hypoxia Symposium promises to be a very special event. Please join us, as dictated by tradition, by the Full Moon on February 18-22, 1997 for our 10th Biennial Celebration of Hypoxia and High Altitude Pathophysiology.

John R. Sutton
Charles S. Houston
Geoffrey Coates
Co-Chairs, Hypoxia 1995



DEDICATION

The International Hypoxia Symposium dedicates the Ninth Biennial Meeting to Carlos Monge C. for his life long contributions to our understanding of humans and animals at high altitude. His studies of high altitude dwellers of the Andean Altiplano have given new insights into adaptation and maladaptation to high altitude. His work has embraced most aspects of physiology, especially renal, hematologic and cardiovascular; using techniques extending from molecular biology to epidemiology. In studying high altitude polycythemia he has unravelled the confounding effects of age and altitude in the predisposition to and pathophysiology of Chronic Mountain Sickness—a condition described by his father and which bears the family name. In recent work he has examined the mechanisms of adaptation of avian hemoglobin to altitude and has continually questioned the genetic and environmental interplay in determining the response of high altitude natives to hypoxia.

Carlos Monge Cassinelli - A Personal View of the Setting and Significance of his Work

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Born Into A Natural Laboratory

Most of us realize that Peru is the site for the bulk of Carlos Monge's career. This is a land like none other on earth. It straddles the equator with waves of the Pacific washing its western shores, with high mountain valleys spawning the headwaters of the Amazon, and with the planet's largest tropical rainforest starting on its eastern borders and extending on to the Atlantic, to the mouth of the Amazon. A unique location defines a unique geography that includes coastal deserts, high mountain valleys, high mountain plains, and finally a sharp transition to classical tropical rainforest. Perhaps most striking of all environmental characteristics of this land is the incredibly sharp profile from coastal near-sea level environments to the valleys and plateaus of the Andean mountains at altitudes ranging from about 3000 to over 4500m. The impact of hypobaric hypoxia at these altitudes is severe enough to negatively influence most humans. Yet within this land there are human populations who have been living under conditions of hypobaric hypoxia for generations; there are other human populations that have lived at altitude for only short periods of time—extending from days, to weeks, to months, to years, but not to generations. Within this land, there are human populations whose residence and living conditions change hardly at all with time, while there are other human populations whose life style dictates continuous movement up and down from near sea-level to the high mountains over very short periods of time. The physiological stresses and adjustments required under these various conditions differ, sometimes dramatically, and exactly the same considerations apply to animals that live in similarly varied environments. To biologists this land is a kind of heaven—filled with fascinating problems of adaptation and evolution of animal and human life in varied environments showing sharp transitions between each other. When Carlos Monge arrived on this earth in the spring of 1921, little did he realize that he was born in the heart of one of the planet's most wonderful natural biological laboratories. Little did he realize that this would affect the rest of his life.

The Basic Background Facts

Carlos Monge Cassinelli was born on September 1, 1921 at a sea level environment—Lima, Peru—where he lived his formative years as a boy and where he pursued most of his career as an adult. His formal training was received at the University of San Marcos (Faculty of Medicine) in Peru and at Johns Hopkins University (Dept. of Medicine) and Cleveland Clinic & F.E. Bunts Educational Institute in the U.S.A.

He held several positions both in Peru and in the U.S.A. during his career; since 1986, he has been at the University of Peruana Cayetano Heredia in Lima. He has deservedly received many honors and awards in his career, including fellowships from the Rockefeller Foundation and from the Guggenheim Foundation in the U.S.A. In 1992, he was honored with the Palmas Magisteriales en el Grado de Amauta in Lima, Peru. Further Curriculum Vitae are available in an interesting little book published on occasion of his 70th birthday⁹. The salient fact of Carlos Monge's career is that for the latter half of this century he has been a towering figure in the field of hypobaric hypoxia research. If one considers his father's work as well, it is clear that the Monges have been dominant forces in the field for the better part of this century! My task, which I accepted an an honor and as a command, is to try and put this enormous contribution into perspective. How does this work fit into the overall scheme of things?

Three Basic Themes in Hypoxia Research

In considering this task, the first thing we must realize is that hypoxia research is an enormous international endeavour. A search of data bases easily available at our libraries recently identified over 20,000 publications in the field during the last 5 year period. Even a cursory examination indicates that three themes are intertwined in this vast literature. One of these, theme (a), focusses on hypoxia-tolerant species and as its main goal aims at uncovering molecular and metabolic mechanisms of defense against hypoxia in species that can survive inordinate O₂ limitation. Studies in theme (a) are basically dealing with 'normal' physiology of species that are 'abnormally' hypoxia—or even anoxia—tolerant. The second theme (theme (b)) focusses more upon hypoxia-sensitive systems in which research is dealing primarily with pathophysiology and the main goal of research is defense, protection, or reversal of the debilitating effects of O₂ limitation. The third, and more recently developing theme (theme (c)) lies at the interface between these two quite distinct research approaches; it aims to explore the possibilities of useful transfer of defense strategies of hypoxia-tolerant systems to more applied situations, potentially as guidelines for more clinically oriented researchers for intervention during O₂ limitation in hypoxia-sensitive systems⁴.

The characteristics of these three intertwining themes in hypoxia research are illuminating. Theme (a) is dominated by a frequent reliance on the August Krogh principle, which was first clearly enunciated by Sir Hans Krebs⁷ in a paper presented on occasion of C. Ladd Prosser's 65th birthday. Krebs argued that there are two tenets to the Krogh principle: namely, (i) that organisms can be used as an experimental parameter *per se* and (ii) that for each major biomedical problem nature has invented the ideal organism for its study. Krebs then went on to supply examples that extended from his own biochemical work on the discovery of the citric acid cycle⁷ across many levels of organization ultimately even to research in animal behaviour⁸. Taking advantage of the Krogh principle in theme (a) hypoxia research thus means using species (organs, tissues, cells) that are especially well suited for analysis of the hypoxia problems being addressed. For example, aquatic turtles are so anoxia-tolerant that they can survive months of complete O₂ lack; they thus become an ideal system for analysis of general anoxia-defense frameworks. High altitude adapted animals such as the Andean camelids are exquisitely designed for function in hypobaric hypoxia; they are thus an excellent mammalian model and have so been used by physiologists

for many decades. Another characteristic of research in theme (a) is its reliance on so-called ‘natural experiments’; this is in fact a common strategy of research in biology, where careful analysis of nature’s experiments can allow insights into hypoxia defense mechanisms that would be otherwise difficult or impossible to set up with ‘normal’ experimental systems. For example, the natural migrations of deep diving seals make it clear to biologists that these organisms can remain submerged for up to and over 90% of the time, that individual dives can reach two hours in duration, and that these organisms can perform this way for weeks or even months at sea at a time. These ‘natural experiments’ thus yield important insights to the adaptation limits and requirements that have to be explained by any viable theory of O_2 management during voluntary diving when the organism may frequently find itself on the knife edge of anoxia. A third and equally important characteristic of theme (a) is a simultaneous integrationist-and-reductionist approach to analysis of hypoxia defense mechanisms: reductionist molecular physiology suggests the ground rules for low O_2 operation while integrative physiology sets the limits and operating conditions for those ground rules. The continuous interplay between these two approaches is synergistic and leads to insights that could not arise from pursuing either approach separately⁴.

Theme (b) is more difficult to summarize. Theme (b) is really the mainstream in hypoxia research. In this area, perhaps 90% or more of the research is done with rats or humans, or with cells, tissues, or organs derived from these species. In trying to achieve the main goals of theme (b)—intervention designed to protect against hypoxia or to reverse hypoxia or reperfusion damage—the main strategy of research almost always includes a strong pharmacological element (for example, see ref. 14). Work here is typically phenomenological, rather than mechanistic, and if the latter, it is typically reductionist. Generally, there is a wide gap between reductionist research in this area and integrative physiology; in fact, workers at the two different ends of the integrative-*vs*-reductionist research spectrum are largely unaware of each other. Similarly, workers in theme (b) typically are largely oblivious of workers or work in theme (a), although there are a few striking exceptions to this statistical rule.

If theme (b) is the mainstream, then theme (c) is best defined as a slip-stream! There are only a few brave souls in this area; however, the number and size of this research area is growing and recently⁴ an International Union of Biochemistry Symposium specifically focussed on the interface between themes (a) and (b); a serious attempt was made to evaluate how much of the classically comparative field of hypoxia research had a bearing on the mainstream and in particular what kinds of defense strategies of hypoxia-tolerant systems could constitute effective framework for intervention strategies in more clinically oriented research. In research papers, it is often difficult to evaluate the status of theme (c). Often the scientists involved are either too close to their own research or too cautious to be concerned with whether or not clinical researchers could use any of nature’s blueprints of hypoxia defense as intervention strategies in clinical settings. In turn clinical researchers may find it difficult to pick through the details of pure science contributions to see what if anything could be transferred to more traditional medical goals. From recent assessments^{3,4} it is clear that several defense mechanisms in hypoxia-tolerant systems are well enough understood to make them ripe for transfer into more applied research settings; many others, on the other hand, are not yet at a stage where they would be particularly useful to more clinically-oriented researchers.

Monge's Approach to Hypobaric Hypoxia Research Shares Many Features with Theme (a)

When I first encountered Carlos Monge and his work in the Andes, I was struck by many similarities between his approach to hypobaric hypoxia research and that of theme (a) above. In the first place, I immediately 'heard' echoes of the August Krogh principle: choosing study systems that are ideally suited to the research problem at hand. For Monge, finding himself in the middle of a natural human laboratory, this was and is a relatively straightforward process. The Andes are filled with such 'best choices' for study systems. Temporal gradients of adaptation and gradients of 'de-adaptation' are especially advantageous. With regard to the former, some indigenous peoples have been living successfully at high altitude for time immemorial; the Quechuas and their forebearers, for example, are generally assumed to have been living in the Andes for over 10,000 years. Some high altitude people have been exposed to altitude hypoxia for only a few generations; some, for less than one generation; and some, for less than a lifetime. On a comparative scale, these different groups constitute ideal study systems. With regard to varying degrees of 'de-adaptation', by the time Monge started his career, the disease of over-expression of red blood cells was already known, named after his father and defined in part as a de-adaptation. This over-reaction to chronic hypoxic stimulus is a kind of centre piece around which many organ and tissue specific hypoxia responses are orchestrated; because it is characterized by upwards regulation of the red blood cell mass, it is a simple matter to find individuals whose hematocrits vary systematically from 'normal' to 'abnormally' high values. On a comparative scale, these then also constitute ideal types of study systems and form the basis for much of Monge's research—from studies of hematocrit effects on basic renal functions to effects on cerebral blood flow. In these kinds of ways, much of Monge's work implicitly or explicitly frequently makes such 'best choices' of study systems for the specific research problems under investigation—a key hallmark of theme (a) hypoxia research.

A second indication of theme (a) are echoes of the 'natural experiment' that so typifies this approach to research in hypoxia. This is really a special version of the above, with emphasis on the 'natural experiment' rather than 'natural system'. For example, Monge's work has often taken advantage (i) of natural movements of peoples between low and high altitudes, (ii) of miners exposed as a result of their work requirements to environmental stresses in addition to hypobaric hypoxia, or (iii) of the 'natural' phylogenetic differences between Andean natives and Himalayan natives, 'naturally adapted' to hypobaric hypoxia for different periods of biological time. Again, this theme (a) characteristic is evident broadly in his work—from comparisons of hematological responses to hypoxia in Quechuas *vs* Sherpas to a comparison of ventilation and its response to chronic hypoxia in lowlanders compared to indigenous highlanders.

Thirdly, there are echoes in Monge's research in hypobaric hypoxia of the theme (a) characteristics of continuous interplay between molecular reductionist physiology and integrative physiology. To be sure, formally Monge is trained in (and my sense is that he tends to prefer) integrative physiology; this explains his numerous contributions to whole-organism level physiology of humans, animals, and even avian eggs under hypobaric hypoxia. However, this has not discouraged him from simultaneously exploring molecular (reductionist) properties of red blood cells, of hemoglobin structure and function, of hormone action, of tissue biochemistry, and of avian eggs. What

is more, he has pulled the intellectual thread through the eye of the needle required to integrate the two approaches and distil from them added physiological meaning^{10,11}. Thus the similarities between theme (a) and the Monge approaches to hypoxia research are everywhere evident.

Monge's Approach to Hypoxia Research Also Shares Features with Theme (b)

As a pure research scientist, I had the greatest difficulty perceiving and accepting Monge's theme (b) of more applied work and interests. This is because I was initially blindsided by the unexpected and impressive theme (a) characteristics of choosing the ideal experimental systems and natural experiments for probing human hypobaric hypoxia responses. Nevertheless, perusal of Monge's career and productivity makes it clear that he is profoundly motivated by the classical theme (b) goals of intervention and protection against hypoxia, or of reversing, the debilitating effects of chronic hypoxia on those individuals who succumb to this environmental stress. Adding to my own initial difficulties at seeing the full range of Monge's theme (b) research in hypoxia is the apparent paradox that this work frequently focusses almost exclusively on what I have already defined as a 'best choice' of study system—the indigenous highlander—but in this case, the highlander who for unknown reasons, succumbs to the unrelenting hypobaric hypoxia of his environment. To Monge, however, the ideal choice of study system probing purely theoretical questions does not preclude a profound classical medical interest in the theory and practice of conquering so-called 'high altitude' diseases¹³. Again, because of his perception that a high hematocrit is a central physiological condition which determines many tissue/organ responses to hypoxia, much of Monge's theme (b) research takes as its point of departure the goal of reversing the harmful effects of excessive polycythemia, while simultaneously recognizing other potential problems that can arise such as CNS complications (from headache to migraine to stroke), compromised renal function, and other hypoperfusion based organ-specific perturbations.

Monge's Approach to Hypobaric Hypoxia Research Also Shares Features With Theme (c)

When I began to analyze Carlos Monge's work, what was perhaps most interesting of all to me was a similarity that I saw between his 'within-species' approach and the more classically comparative 'between-species' approach to hypoxia research. In addition, implicit in all of Monge's work with humans indigenous to high altitude is the assumption that much of their response is the 'healthy' human's response to hypoxia; only in the extreme case (for example, in Monge's Disease or perhaps in old age) is the response that of an 'unhealthy' human. Interestingly, there is an enormous dearth of information in theme (b) research on the 'healthy' response to hypoxia challenge, a serious, near-fatal flaw in the theme (b) approach to hypoxia research (Monge's work here being a notable exception). Theme (b) if anything is overwhelmed by disease syndromes¹⁴—by heart disease and cardiac arrest, by similar disease in the CNS and stroke, by acute renal failure, by liver ischemia, and so forth. None of this research can tell us anything about how the normal or healthy organ or system would protect itself against hypoxia. Hence, implicit in much of Monge's work on indigenous highlanders¹¹ is the theme (c) idea of potential transferability of hypoxia defense strategies of healthy humans to more traditional clinical conditions. Implicit in his work on hypoxia adaptations in animals is the very same concept. In fact, I have

argued elsewhere³ that the main Monge legacy in human hypoxia research challenges us to evaluate how hypoxia defense mechanisms of adapted systems (animal or human) can be harnessed as intervention strategies or frameworks in protecting more hypoxia-sensitive systems against O₂ limitation.

Why the Enormous Influence of Monge's Work

Whereas this theme (c) derived legacy challenges and focusses our future research interests, in itself it does not account for the enormous impact of Monge's contribution. In fact, there may be no single, simple explanation for the latter. But if there is, in my opinion it must be because Monge's work overlaps and subsumes all the three major themes which have been evolving in the field of hypoxia research over this century. In the enormous literature to which I refer above, most of us work within rather limited vistas; individuals like myself mainly dabble with theme (a) approaches. Most of my medical colleagues in North America work purely within the theme (b) framework. Judging from the only assessment thus far carried out⁴, only a few of us work in theme (c); the area is still minute and is easily overlooked. In contrast, Monge's work is at once (i) pregnant with information on perhaps the best choice of study system available for unravelling the effects of chronic hypoxia on human physiology and biochemistry—theme (a); (ii) it is filled with insight into intervention strategies again based on ideal systems for study-theme (b); and (iii) it is anxious to inquire into which adaptive features of the 'best choice' of study system can be transferred to defending more hypoxia sensitive tissue/organs or individuals in more traditional clinical settings—theme (c). The secret of Monge's enormous success may well lie in the fact that, unlike most of us, he towers over all three major themes in late 20th century hypoxia research.

Given this stature, one might wonder if there is any area of human hypoxia research that eludes the Monge reach. Are there any issues so difficult and stubborn that not even the Monge three-pronged attack can solve? Perhaps surprisingly, given the evident Monge success in many areas of human hypoxia research, the answer is affirmative.

Monge's Final, Big and Nagging Questions: Extent and Limits of Human Adaptability

As a biologist, I am most fascinated with two additional unresolved issues that are found running throughout the enormous research output of both senior and junior Monge; namely, the problems of extent and limits of human adaptability to hypobaric hypoxia^{2,10,11}. These issues of course collapse into the single unsolved problem of human adaptability which transcends their work and actually pervades the entire hypobaric hypoxia literature. Thus to put this area into perspective, it is important to emphasize that one reason for the problem being so seemingly intractable is confusion stemming from definition. To physiology, a trait is defined as an adaptation if it can be demonstrated to be 'useful' in performing some organ, tissue, cell, or molecular function and thus to aid in survival of the organism. To biology, however, this is usually considered an inadequate definition. To biology, a trait is an adaptation if and only if it is the outcome of focal selection; in this context, many so-called adaptations are merely characteristics, whose origin is obscure and whose origin may well have involved a coincidental linkage with some truly adaptive trait. Demonstrating a true

adaptation in this context becomes a rather daunting proposition; with humans, it borders on the impossible.

Monge's Approach to the Adaptation Issue

In his characteristic and systematic way, Carlos Monge has probed the issue of human adaptation to hypobaric hypoxia in two ways: using 'between-species' and 'within-species' analyses to throw light on the problem^{9,10,11}. The conclusions from the first approach—comparing animal adaptability limits to those of humans indigenous to high altitude—are rather disappointing for on this scale of comparison humans show almost imperceptible and certainly immeasurable hypobaric hypoxia adaptations. The functional properties of hemoglobins supply the textbook example of the rather puny hypoxia adaptation potential of the human species compared to high altitude adapted animals: in most, perhaps all, examples of the latter, hemoglobin affinity for O₂ has been scaled upwards while hemoglobin concentration and hematocrit have been scaled downwards. Judging from successful high altitude animals, the most effective hypoxia adaptation strategy for this level of physiology clearly is high efficiency of O₂ loading at the lung and low cost of red blood cell transport through the blood (low viscosity). No humans—not Sherpas, not Tibetans, not Andean natives—no humans known today anywhere on this planet have been able to achieve this adaptation to hypobaric hypoxia. On several similar criteria that have been analyzed^{1,2}, the same conclusion arises: on interspecies scales of hypobaric hypoxia adaptation, humans display minimal, essentially immeasurable adaptation potential.

However, the Monges have also probed the issue of human hypoxia adaptability limits by means of 'within-species' comparisons. The classical comparisons are of humans adapted to high altitudes for differing amounts of phylogenetic time—the commonest comparisons are of lowlanders, Andean natives, and of Himalayan natives. On this scale, many physiologists and biochemists accept that there may be some evidence for true hypoxia adaptations within the human species. One example that Carlos Monge has helped us to expose concerns the nature of heart metabolism in high altitude natives. Both Quechuas from the Andes⁶ and Sherpas from the Himalayas⁵ have been studied, but currently the best evidence probably arises from the Sherpa studies. Magnetic Resonance Spectroscopy studies of heart metabolism suggest very striking differences between Sherpas and lowlanders that are seemingly stable for at least 3-4 weeks following de-acclimation from high altitude. The differences noted are consistent with a metabolism in the heart of altitude natives designed to use oxygen 25-60% more efficiently than in the heart of lowlanders⁵. Thus, as originally proposed by the senior Monge¹², and periodically considered by the junior Monge¹¹, there are tantalizing bits and pieces of evidence suggesting true biochemical and physiological 'defense' adaptations of high altitude humans to hypobaric hypoxia. Indeed, it is interesting that Chronic Mountain Sickness or Monge's Disease was originally defined as involving a 'loss of adaptation' to hypobaric hypoxia^{11,12}. Be this as it may, it is important to acknowledge that gene or protein sequence data providing evidence of focal selection for specific advantageous functional adjustments are thus far lacking—and until such data are forthcoming, we must maintain an open mind (as indeed Carlos Monge has done through most of his career) on the issues of the extent and limits to human hypoxia adaptability. Compared to animals, we know the potential is small; compared to lowlanders, indigenous highlanders may indeed express some true biological adaptations, but proof of this contention is not yet definitive.

Doing Competitive Science in a Developing Country

The above thumb-sketch hopefully gives the reader a sense of how, born in the heart of a natural biological laboratory, Carlos Monge took advantage of the situation (using best choices for the problems under study and using natural experiments which would be impossible for an experimenter to directly set up or even mimic) to make an enormous impact internationally on the field of hypobaric hypoxia research. To many of his colleagues around the world, however, one of the most impressive aspects of Monge's career is remaining so productive and so competitive in a fast-moving and vast field of research while doing much (indeed, most) of his research in a developing country. As many of us who have either worked in the Andes or areas like it elsewhere in the world know too well, this is no easy or simple feat; it is an awesome achievement, in its own right deserving special mention and special accolades. I well understand that many of Carlos Monge's colleagues in the Andean nations properly appreciate this special and unique contribution and have awarded him recognition and honor for this reason. That presumably is the main meaning of his 1992 *Palmas Magisteriales en el Grado de Amauta*.

Realizing that one literal translation of *Amauta* is 'sage of the Incas', on my last trip to the Andes, while visiting Machu Picchu, I imagined the possibility of Peru offering him a different, and perhaps even higher, kind of honor and recognition: the remoulding of a part of that most noble of Inca images, Huayana Picchu, and renaming it—*Monge Picchu*! In my mind, that could constitute Peru's ultimate 'homage' to one of its finest sons.

Carlos Monge Cassinelli is not only 'sage of the Incas'; he is 'sage' of a large and admiring international community of scholars, scientists, and medical doctors, who will remember his work forever.

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HOW THE HYPOXIA SYMPOSIA BEGAN

Like many babies, it is not certain exactly when conception of the Hypoxia Symposia occurred. Like most human endeavors it is the fruit of many persons' work and dreams. Yet, to be as precise as possible, we might date the origin of these (and their younger siblings) to the fall of 1973.

High altitude mountaineering was flourishing by then and many of the highest summits had been reached without supplemental oxygen. It had become obvious that there were many hazards in mountaineering which climbers—and doctors—knew little about and were unprepared to manage. Charlie's experience in the high Himalayas dated back to 1936 with the climb of Nanda Devi, and his interest had been refueled by a case of high altitude pulmonary edema he saw in Aspen in 1961.

Believing that some of the early Everest climbers had valuable knowledge about altitude to share, Charlie wrote old friends in England, and in the fall of 1973, with help from the Alpine Club, gathered a dozen of the great British mountaineers for an afternoon of discussion in London. Among them were Noel Odell, Bill Tilman and Raymond Greene to talk about their personal experiences on the highest mountains.

Michael Ward chaired the meeting and set the stage with a summary of what little was known then about the physiology of altitude illnesses. Though no written record survives, the discussion was so stimulating that the Alpine Club arranged a larger and more formal meeting for March 1975 in Wales, the proceedings of which were published a year later¹.

In July 1975, the eighth year of the high altitude research on Mount Logan², many of the support team who served as subjects for the research were or planned to be medical students. John Sutton and Peter Powles gave them several talks about various aspects of altitude physiology and medicine while they were getting their baseline studies at McMaster University. Later at Base Camp on Kluane Lake in the Yukon, John led more extensive seminars for the subjects and the project scientists. These people were enthusiastic about these talks, suggesting that other mountaineers and scientists might be interested too.

So Charlie persuaded the Yosemite Institute to sponsor a three-day meeting in October 1975 in Yosemite National Park. At this nervously planned meeting many aspects of mountain medicine were discussed by experts in altitude, cold injury, trauma, medical evacuations and other topics of importance to climbers, trekkers and casual visitors to the mountains. This Mountain Medicine One was so successful that three more were held, one in Yosemite and two, sponsored by the Arctic Institute, in Banff, the immediate ancestors of our present symposia.

The objectives were well defined: to offer the best available information about mountain hazards not only for doctors but specifically for climbers, hikers and any others who might be in harms way in the mountains. Each attracted a large audience. Authorities like Noel Odell from England, Bo Siesjo from Sweden, Jacques Foray from France and other veterans spoke, and many others presented abstracts of their work for the medical audience.

Then in 1978 Everest was climbed, *au naturel* as it were, and as more climbers tried the high mountains, more deaths from altitude illness were recorded, and another Mountain Medicine meeting was held in 1979. Although one of the four days was designed for mountaineers and covered many mountain hazards besides altitude illnesses, the main thrust of the meeting was on research in how oxygen was transported and used. This meeting was held in Banff and the name Hypoxia Symposium and the logo were used for the first time.

Of historical interest are the misgivings which clouded this decision. Some said that mountaineers wouldn't come; others warned that true scientists were already committed to more esoteric meetings. Many doubted that there would be enough new material to attract the best scientists. But almost immediately these fears proved groundless: people did come, and outstanding scientists and climbers eagerly accepted an invitation to speak. We were determined to keep the cost modest, but even so the only real problem (which turned out to be perennial) was lack of money!

The programs got better and better, more and more world authorities came to listen or talk, and more and more junior scientists submitted good research abstracts. Subsequent meetings have been designed for scientists exploring all aspects of hypoxia—due to altitude or illness, in man as well as in birds, fish, insects and mammals.

These scientific presentations are sophisticated, cutting edge stuff, but nevertheless, respecting the origin of the meetings, one day of each Symposium is devoted to Mountain Medicine, including clinical cases and practical advice for mountaineers.

Recently mountain medicine has become fashionable and other groups discuss trauma, rescue, avalanches and many risks in the mountains. At our Symposia the Day in Mountain Medicine stresses altitude hypoxia and its effect on those who go to the mountains for work or play. Many climbers come for this day alone while some try to digest the program on other days as well. Many scientists who are not mountaineers attend the Mountain Medicine talks looking for clues to relevance for their research. The easy access to great outdoor winter sports does not detract from or distract the audience.

In 1982 the Proceedings of the second Symposium (1981) were printed, as they have been for each one since then³⁻¹⁰. Though most of the books are out of print, some of the more recent ones are still available.

Many senior scientists have attended the Symposia over the years, including Griff Pugh, Hermann Rahn, Hugo Chiodi, Bruce Dill, and Ulrich Luft. Distinguished mountaineers like Barry Bishop, Kitty Calhoun, Peter Hackett, Oswald Oelz, John Roskelley, Michael Ward, and Jim Wickwire have spoken at the evening sessions.

A special feature, started in 1989, has been dedication of the meeting to an individual who has devoted his or her lifetime to improving what we know about hypoxia and mountaineering hazards. Those honored include Herman Rahn, Griff Pugh, Carlos Monge, and began with Charlie Houston in 1989.

Meanwhile, since 1975, many other meetings dealing with the mountain environment have evolved. Most are clinically oriented to provide practitioners with continuing education in this new specialty. The oldest of these, the Mountain Medicine Institute in Oakland, California¹¹, presents six to eight weekly lectures each year on all aspects of outdoor medicine. These have attracted more than 16,000 people since 1975. The Wilderness Medical Society¹², dedicated to education, has been sponsoring

longer meetings since 1986, often holding two meetings each year. Many others are commercially sponsored.

It is nearly a quarter century since our Symposia began and the quality of the program and enthusiasm of the audience continue to increase. With appropriate modesty we may say that these Symposia have fathered many offspring, regardless of when or how they were conceived.

Some have asked if we will continue. . . . The answer is emphatically YES, and during the first full moon in February every other year. We have reserved that time at the Chateau Lake Louise until 2001. Why the full moon? Well, not for the same reason that the Lunar Society met in Birmingham every month for fifty years. Nor, to quote Mallory, "Because it's there." Simply because it is so beautiful.

John R. Sutton

Charles S. Houston

Geoffrey Coates

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CHAPTER 1

CNS RESPONSE TO HYPOXIA

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Introduction

The functions of the brain altered by hypoxia are well known. At sea level, when the PO_2 is decreased to 75% of normal, complex task performance is altered; at 65% short term memory is impaired; at 50% judgment is altered; unconsciousness occurs with PO_2 's between 30 and 40% of normal³. Death from hypoxia was recognized as early as 1874, when three balloonists lost consciousness at an altitude above 23,000 feet; two of the three men died¹⁹. At high altitude the impact of hypoxia upon climbers appears to be on cognitive more than motor skills^{12,7}. Whether or not permanent residual nervous system abnormalities occur is controversial, but a statistically significant decline in digit recall (regarding cognitive function) and speed of finger tapping (representing motor function) has been found following an expedition to the summit of Everest; the abnormalities lessened but at 12 months after the expedition still remained statistically different than the pre-expedition testing^{21,14}.

Background

The classic experimental literature, however, does not support hypoxia as an etiology for neuronal death in brain. The primate experiments by Brierly *et al*, 1978⁵ used 3.2 percent oxygen in a nitrogen atmosphere as hypoxic exposure. Brain damage was rare in these animals, using neuropathology as the criterion. When brain damage did occur, selective neuronal loss was not found but rather cell death and/or infarction was found in arterial border zones and therefore was the result of superimposed ischemia. A similar series of experiments was reported by DeCourten, Meyers, *et al*, 1985⁹. Cats were exposed to a PaO_2 of approximately 17 mmHg for 25 minutes. Following this exposure to hypoxia, the cats were normal clinically and neuropathologically, unless hypotension supervened. With hypotension, again, border zone infarction occurred. Chemical hypoxia has also been studied by Brierly *et al*, 1977 and MacMillan, 1989^{4,13} using cyanide exposure *in vivo*. Little neuropathologic injury occurred if systemic pressure was maintained.

Some clinical data in humans also support the relative benignity of profound hypoxia. A group of 22 patients was studied at the Yale New Haven Hospital because of PaO_2 's less than 20 mm/Hg (the lowest 7.5 mm/Hg). Of the 22 patients, 13 recovered despite PaO_2 's below 20 mm/Hg. Those recovering included the patient with the lowest PaO_2 and both patients who were comatose with decerebrate rigidity¹¹. Some

Abbreviations: AMPA, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; NBQX, 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(f)quinoxaline; NMDA, N-methyl-D-aspartate; SDH, succinate dehydrogenase.

neuropathologic data from humans is available as well. Three young patients, 16 to 19 years of age, were previously healthy but developed acute respiratory hypoxia with PaO_2 's below 45 mm/Hg which persisted for one to eight days prior to sudden fatal cardiac failure. Just prior to death PaO_2 measurements were 24 to 38 mm/Hg and arterial pressure measurements were 55 to 80 mm/Hg. Detailed neuropathologic examination showed no evidence of anoxic-ischemic injury in any of the brains¹⁵.

The mechanisms by which neurons die in an hypoxic atmosphere have been substantially clarified over the last decade in both *in vivo* and *in vitro* systems⁶. The initiating phenomenon appears to be an elevation in the concentration of the excitatory amino acid neurotransmitter glutamate within the extracellular compartment. Glutamate induces depolarization of the post-synaptic membrane via the AMPA gated channel. Such depolarization opens voltage-gated and receptor-operated calcium channels. Continual glutamate exposure, therefore, results in intracellular calcium toxicity which induces catabolic processes within the cell, ultimately producing protein denaturation and cell death. The potential neuronal injury can be detected even at the early stages, as glutamate concentrations in the extracellular compartment can be monitored by *in vivo* microdialysis techniques². Further, early and subtle neuronal injury can be detected immunocytochemically by the use of antibodies to nonconstitutive heat shock proteins (HSP) which are induced by a wide variety of stresses in the brain, including ischemia¹⁶. Induction of HSP's represents a response of the cell to the presence of denatured protein¹. The presence of denatured proteins activates heat shock factors²² which bind to heat shock elements which result in transcription of HSP RNA^{23,24}. Thus, neurons which stain immunocytochemically with HSP antibody contain denatured proteins as evidence of early intracellular injury.

We therefore used the highly sensitive techniques of *in vitro* microdialysis and HSP immuno-cytochemistry to examine the role of pure hypoxia, in the absence of ischemia, in the production of brain injury.

Experimental Design

Paralyzed ventilated normothermic adult male Sprague Dawley (approximately 350 grams) rats were studied. Monitoring included femoral arterial pressure and EEG. A microdialysis probe was implanted in the brain region most vulnerable to hypoxic-ischemic injury, the dorsal hippocampus, and cerebral oxygen content was continually monitored by a thin film oxygen probe (Ottosensors) also placed in the hippocampus. Following standardization of the monitoring parameters, 2% halothane and 24% oxygen with the balance nitrogen was changed to 6% oxygen with the remainder nitrogen, and the halothane was discontinued. Arterial blood pressure was maintained above 60 mmHg using intravenous boluses of normal saline as necessary. After 20 minutes of 6% hypoxia an FiO_2 of 24 to 30% was delivered for a 40 minute recovery period.

For comparison, oxygenated animals (FiO_2 of 20 percent) were prepared and subjected to global ischemia via 4 vessel occlusion techniques for 20 minutes. The duration of global ischemia was confirmed by EEG isoelectricity¹⁶. All animals were permitted to recover and were sacrificed 24 hours later. The brains were prepared for immunocytochemical staining; glutamate concentrations in the microdialysate were measured using HPLC¹⁰.

Results

The effects of hypoxic ventilation on arterial blood gas values and mean arterial pressure (MAP) are found in Table 1. Figure 1 illustrates the changes in extracellular

fluid oxygen tension in the hippocampus of rats given 20 minutes of 6% inspired oxygen (open diamonds, n=3) or 20 minutes of four vessel occlusion (solid diamonds, n=3). Bars indicate standard error. Bullets indicate values significantly different between groups at given time points using the non-parametric Mann-Whitney rank sum test; p=0.05. Figure 2 illustrates the changes in brain microdialysis concentrations of glutamate in rats given 20 minutes of 6% inspired oxygen (open diamonds, n=5) or 20 minutes of four vessel occlusion (solid diamonds, n=3). Bars indicate standard error. Bullets indicate values significantly different between groups using the non-parametric Mann-Whitney rank sum test; p=0.05.

Table 1.

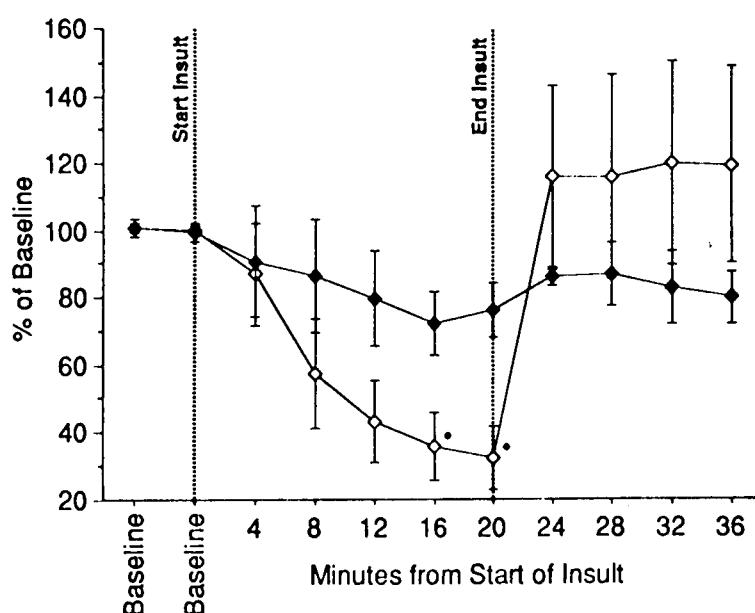
	Baseline	5 Min	10 Min	15 Min	Post-insult
pH	7.420±0.029	7.338±0.057*	7.234±0.062*	7.152±0.078*	7.052±0.4*
pCO ₂ (mmHg)	43.2±4.2	30.9±3.5*	27.0±3.6*	25.1±3.5*	50.7±3.9*
pO ₂ (mmHg)	87.6±12.8	20.4±2.3*	22.7±3.0*	23.7±2.9*	77.0±10.2*
MAP (mmHg)	103.3±4.0	106.7±23.5	105.7±35.6	99.0±15.7	109.3±11.6

Values expressed and mean + SD

*p<0.01 for difference from baseline

+p<0.001 for difference from baseline

Brain Oxygen Tension



- Indicates values are significantly different from each other using the non-parametric Mann-Whitney rank sum test, p=0.05.

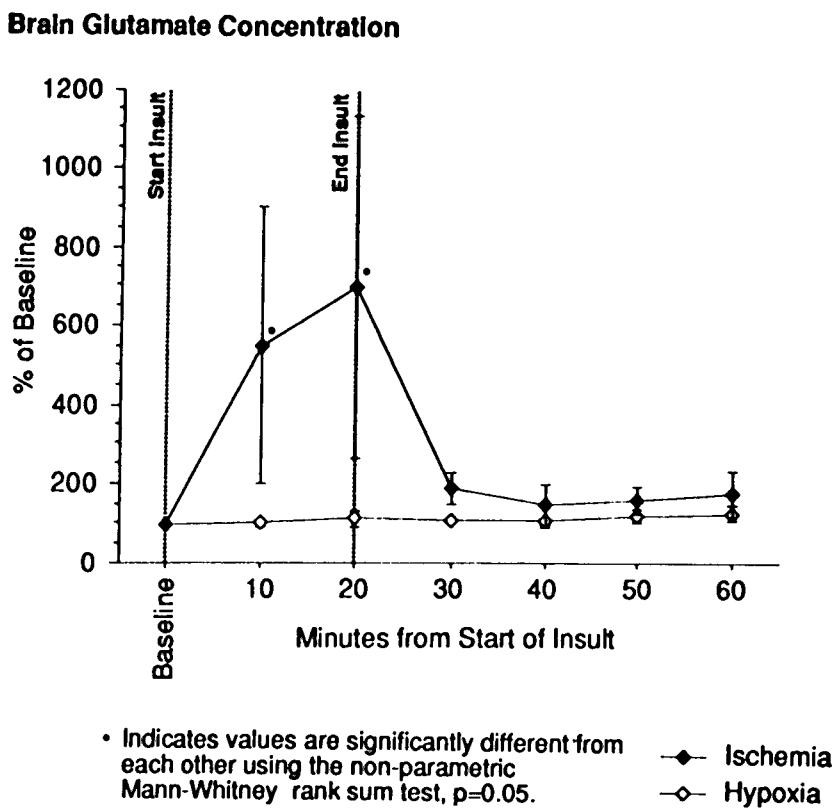


Figure 2

Figure 3 illustrates the immunocytochemical data. Figure 3a shows the effect of 20 minutes of hypoxia upon HSP induction. This section is not different from that of a control animal. Twenty minutes of ischemia however, does induce HSP in the pattern shown in Figure 3b, with diffuse staining throughout the cortical mantle and hippocampus. Figure 3c is a high-power view of the cortical mantle of the ischemic brain.

Discussion

Thus, hypoxia, to the degree obtainable *in vivo* (approximately 20 mm/Hg), does not injure brain neurons, as evidenced by the lack of stress protein induction, nor does it demonstrate the neurochemical signature of *in vitro* hypoxia or *in vivo* hypoxia-ischemia: elevation of extracellular glutamate concentration. This finding does not necessarily mean that an increase of glutamate release did not occur, as astrocytes maintain glutamate uptake during hypoxia or even anoxia as long as there is adequate glucose substrate¹⁸.

These experiments, and those reviewed earlier, studied pure alterations in oxygen content. In the clinical situation there is an additional component of the CO_2 content. In acute ventilatory failure PaCO_2 is elevated, but climbers at altitude have marked hyperventilation with arterial PaCO_2 's reduced to the range of 11 mmHg¹⁴. As has been recently demonstrated that hydrogen ions gate the major receptor-gated calcium

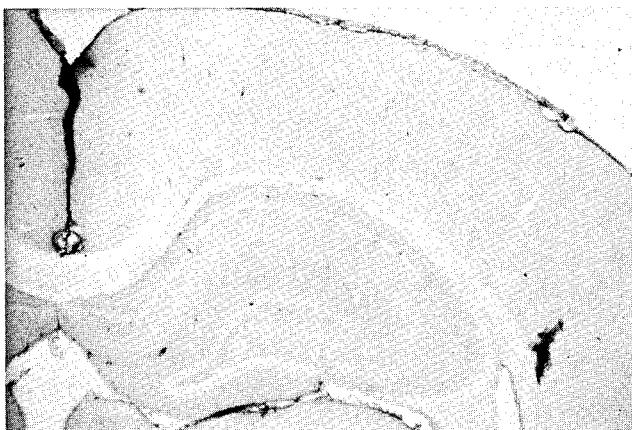


Figure 3a

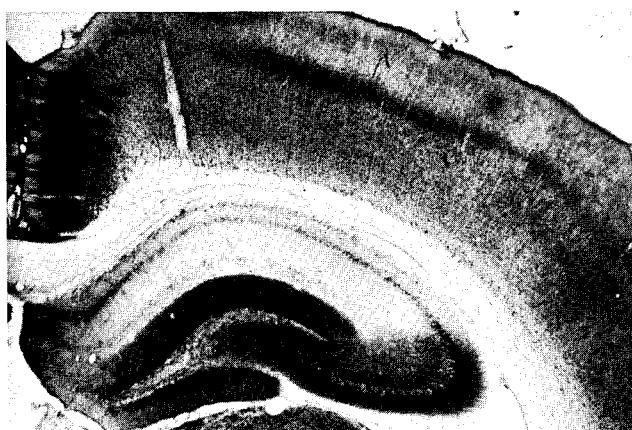


Figure 3b

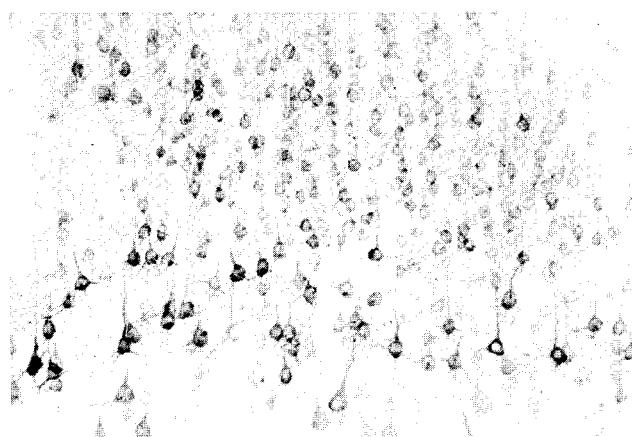


Figure 3c

channel²⁰ (the NMDA-preferring subset of the glutamate receptor), the changes in extracellular pH caused by marked alterations in PaCO₂ could conceivably effect hypoxic brain injury. Hypercarbia and induced respiratory acidosis would tend to be protective. The very high PaCO₂s seen in the hypoxic survivors reported by Gray and Horner¹¹ might be an example of such a phenomenon. In focal ischemia, hypercarbic ventilation has been shown to attenuate infarct size¹⁷. Hypocarbia with respiratory alkalosis, on the other hand, might make the brain more vulnerable to hypoxia²⁰.

What then is the mechanism of hypoxic brain injury? Presumably, such brain injury is due to superimposed ischemia, with a well known and reproducible pattern of injury affecting the so-called selectively vulnerable neurons in hippocampus and cortex. Damage to these cells could be responsible for short-term memory impairment and slowed motor function found in climbers to the Everest summit. Hypoxia (PaO₂s between 15 and 40 mmHg) when confined to the cerebral circulation produce hyperpnea, bradycardia, and cardiac failure⁸. Of the proven effects of hypoxia upon the brain, such induced ischemia is the only clear culprit in the induction of "hypoxic" brain injury.

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CHAPTER 2

MALONATE INHIBITION OF SUCCINATE DEHYDROGENASE: A MODEL OF CHEMICAL HYPOXIA

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Abstract

In this article, we review the neurotoxicity of the succinate dehydrogenase inhibitor malonate. Intracerebral stereotaxic injection of malonate in rats produces dose-dependent lesions that are similar in character to hypoxic/ischemic damage; most neurons are destroyed, but tissue architecture, glia, and certain neuronal populations are largely spared. These lesions are prevented by NMDA antagonists, but are not affected by non-NMDA antagonists or hypothermia. In addition, malonate exacerbates the neurotoxicity of direct excitotoxins, including NMDA, AMPA, and glutamate. We conclude that malonate neurotoxicity provides a simple model of chemically-induced hypoxia.

Introduction

The relationship between neuronal bioenergetics and the glutamatergic system may have profound implications for the pathogenesis of both acute and chronic neurological diseases^{1,5,30}. Although relatively subtle bioenergetic defects may predominate in chronic neurological disorders, the metabolic dysfunction resulting from acute insufficiency of oxygen or glucose is dramatic. This metabolic compromise has a variety of neurochemical sequelae, including a prominent rise in extracellular concentrations of excitatory amino acids^{6,33}. It is hypothesized that these high glutamate levels cause excessive glutamate receptor activation and subsequent excitotoxic neuronal death, but high ambient glutamate levels may also be toxic in other ways¹³. In addition, the postsynaptic manifestations of metabolic impairment may play an equally important role in the acute neurodegeneration accompanying hypoxia/ischemia inso-

Abbreviations: AMPA, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; NBQX, 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(f)quinoxaline; NMDA, N-methyl-D-aspartate; SDH, succinate dehydrogenase.

far as recent experimental evidence suggests that metabolic compromise predisposes neurons to glutamate receptor-mediated excitotoxicity^{3,19,34,39,40}.

There are several mechanisms by which metabolic inhibition may enhance neuronal sensitivity to extracellular glutamate. Metabolic dysfunction impairs the ability of neurons to maintain resting membrane potential by disrupting ion pump function. The resultant depolarization may relieve the voltage-dependent magnesium blockage of the N-methyl-D-aspartate (NMDA)-subtype of glutamate receptor, allowing for easier receptor activation and greater ion flux through the associated ion channel²⁷. This excessive inward ion current (Na⁺ and Ca²⁺) leads to subsequent neuronal death. This process has been termed "weak" or "secondary" excitotoxicity^{1,5}. Systems downstream from synaptic receptors may also be affected by metabolic impairment. These may include second messenger pathways, intracellular calcium buffering, osmotic regulation, and free radical containment. Disruption of any of these systems may render neurons more vulnerable to an excitotoxic insult.

Malonate is a reversible, competitive inhibitor of succinate dehydrogenase (SDH), an enzyme which plays an integral role in both the citric acid cycle and the electron transport chain. *In vivo* malonate injection represents a model of focal, chemically-induced hypoxia. Brain lesions caused by malonate are similar in character to those caused by hypoxia/ischemia or direct excitotoxin injection^{17,20}. This article reviews results from recent experiments in which we used local stereotactic injection of malonate to investigate interactions between bioenergetics and excitotoxicity¹⁷⁻¹⁹.

Methods

Stereotaxic surgery and tissue preparation. All animal use procedures were in strict accordance with the NIH *Guide for the Care and Use of Laboratory Animals* and were approved by the University Committee on Animal Resources. Male Sprague-Dawley rats (200-300g) were anesthetized with 4.0 ml/kg Chloralpene [chloral hydrate (42.5 mg/ml) plus pentobarbital (8.9 mg/ml)] and placed in a stereotaxic frame. Two microliters of solution (pH 7.4) were injected into the right striatum (0.7 mm anterior, 2.8 mm lateral, and 5.0 mm ventral with respect to bregma and dura) or hippocampus (3.6 mm posterior, 2.0 mm lateral, and 3.1 mm ventral) using a Hamilton 10- μ l syringe (26G). Following a 5 minute interval to allow for complete diffusion of the injected volume, the needle was retracted; burr holes were filled with gel foam and wounds were clipped. Animals receiving systemic drug treatment received two injections: one 30 min before surgery and one 210 min after; control animals received bacteriostatic saline. The rats were killed three days after surgery, and the brains were frozen immediately on dry ice. Frozen serial sections (25 μ m) throughout the scope of the lesion were cut and mounted on polylysine-coated slides.

Core temperature measurement. In some experiments, animal core temperature was measured using a rectal temperature probe inserted to a consistent level. Temperature measurements were made immediately following malonate injection and every hour for eight hours; a final measurement was taken 24 hours after surgery.

Cytochrome oxidase histochemistry and analysis of lesion size. Every fourth section was stained for cytochrome oxidase activity. Slides were placed in incubation medium [5 mg cytochrome c and 30 mg 3,3'-diaminobenzidine in 50 ml of 0.1 M phosphate buffer, pH 7.4] for 90 min at 37°C and then removed to 4% neutral, buffered, paraformaldehyde for 10 min. Sections were rinsed with distilled water, dehydrated, cleared in xylene, and coverslipped. The lesioned area on each section was

quantified using a video-based MCID image analysis system (Imaging Research, St. Catharines, Ontario, Canada). The sum of the individual area measurements was multiplied by intersectional distance (100 μm) to determine total lesion volume.

Morphological analysis. Several representative sections were stained for Nissl substance using thionin. Sections were examined under the light microscope, and some were digitized for presentation using the image analysis system.

Chemicals and drugs. The competitive NMDA antagonist LY274614 was a gift from Dr. Paul Ornstein (Eli Lilly, Indianapolis, IN). The competitive α -amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) antagonist 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(f)quinoxaline (NBQX) was a gift from Dr. L. Turski (Schering AG). All other chemicals and drugs were obtained from commercial sources.

Statistical Evaluation. Comparisons between multiple groups were made with one-way ANOVA, followed by post-hoc two-tailed Dunnett procedure or two-tailed Bonferroni-Dunn procedure. Comparison between two groups was made by an unpaired two-tailed t test. A p-value of less than 0.05 was considered significant.

Results and Discussion

Malonate lesions have been most completely described in striatum. They are dose-dependent and involve marked neuronal loss^{4,17,18}. The cytoarchitecture of the striatum remains largely intact inasmuch as axon bundles appear undamaged and the striatal tissue maintains its volume and integrity, at least at early time points. Furthermore, glial cells are largely unaffected by malonate, with the exception of a variable amount of reactive gliosis at the lesion core¹⁷. It has also been reported that, although the majority of neurons are destroyed by malonate injection, specific subsets of neurons are spared. Medium, aspiny, somatostatin/NADPH diaphorase-positive neurons are relatively preserved, as are large, cholinergic neurons²⁰. GABA/substance P neurons appear to be the most susceptible to malonate⁴. Malonate lesions are similar to those seen following transient striatal hypoxia/ischemia, which are characterized by neuronal loss with sparing of glia and tissue architecture, and sparing of somatostatin/NADPH-positive and large, cholinergic neurons^{14,37}. To support the contention that malonate exerts its toxic action by inhibiting SDH, co-injection of excess succinate prevents neuronal damage¹⁸, and injection of malonate markedly decreases striatal ATP and increases striatal lactate⁴.

We have investigated the involvement of glutamate in malonate toxicity by examining the effects of glutamate antagonists or agonists on the volume of malonate-induced striatal lesions. As can be seen in Figures 1 and 2b, the noncompetitive NMDA antagonist MK-801 prevents the neurotoxicity associated with intrastratal malonate injection¹⁸. MK-801 is similarly effective at preventing the toxicity of higher doses of malonate (2 μmole vs. 1 μmole)^{4,17}. These results indicate that the majority of malonate neurotoxicity is mediated indirectly by the NMDA receptor.

One concern as to the specificity of these results is reports indicating that MK-801 induces hypothermia in certain experimental paradigms^{9,12}. Since, in some hypoxic/ischemic models, even small differences in temperature have been associated with large differences in lesion size^{19,11,41}, we measured core temperature and lesion volume in control animals, MK-801-treated animals, and animals made hypothermic by the administration of excess anesthesia (Fig. 2). In this model, MK-801 did not induce any degree of hypothermia at any time point. In fact, its administration was associated with significantly higher core temperatures from 3 to 8 hours after surgery.

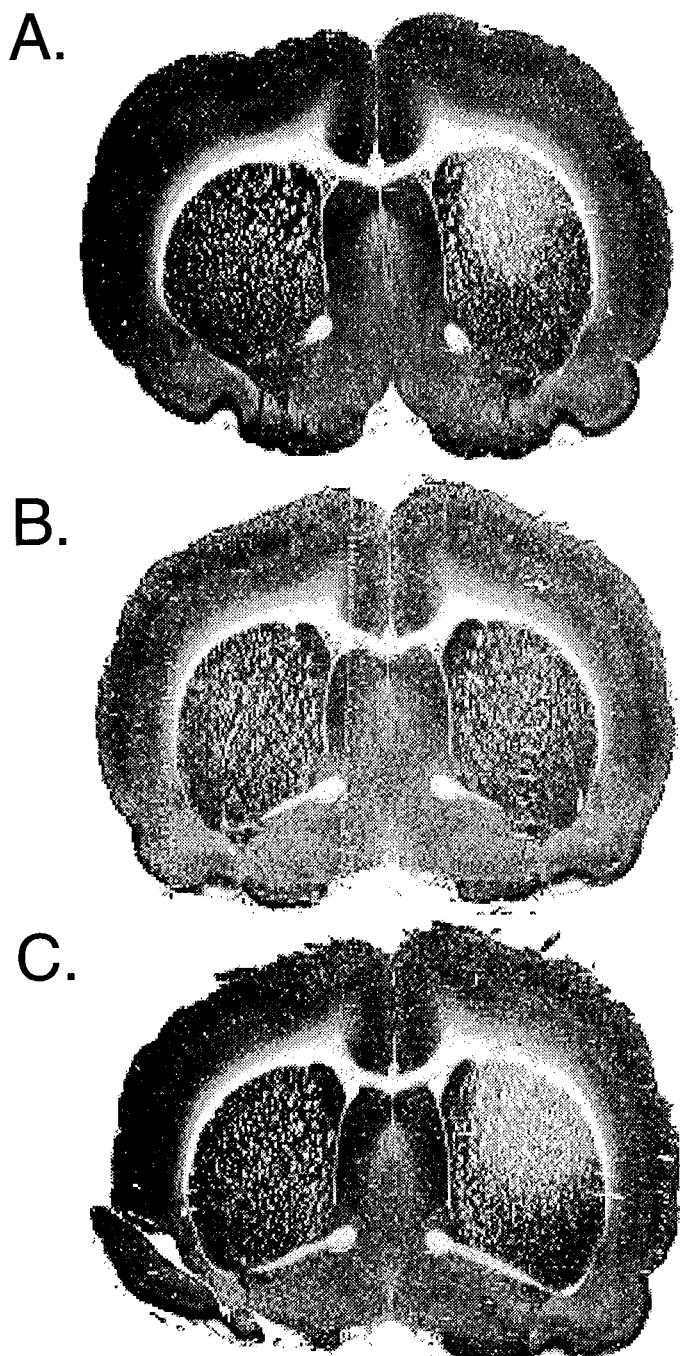
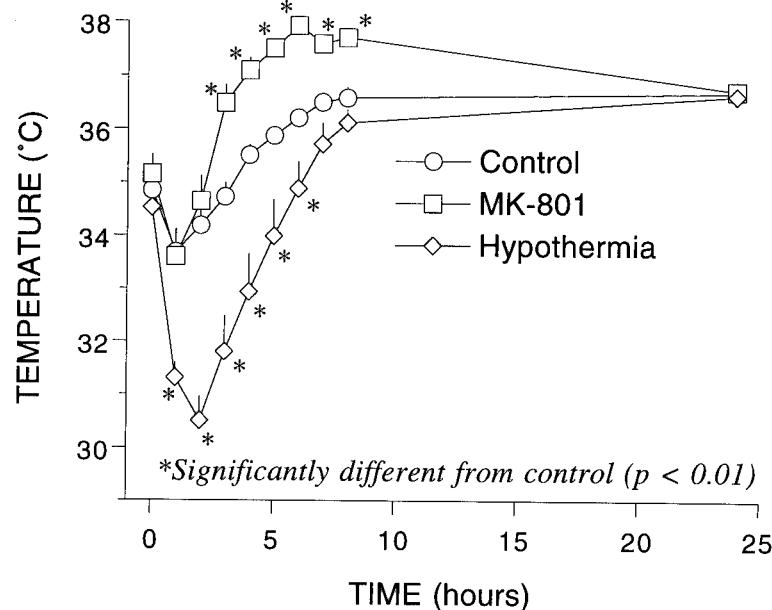


Figure 1 Digitized images of representative cytochrome oxidase stained sections from brains of rats injected with $1 \mu\text{mol}$ of malonate. (A) control, (B) MK-801-treated (5 mg/kg, i.p.) (C) NBQX co-injection (2 nmol) Note the protection by MK-801 and lack thereof by NBQX. *Reproduced with permission from the Journal of Neurochemistry.*

A.



B.

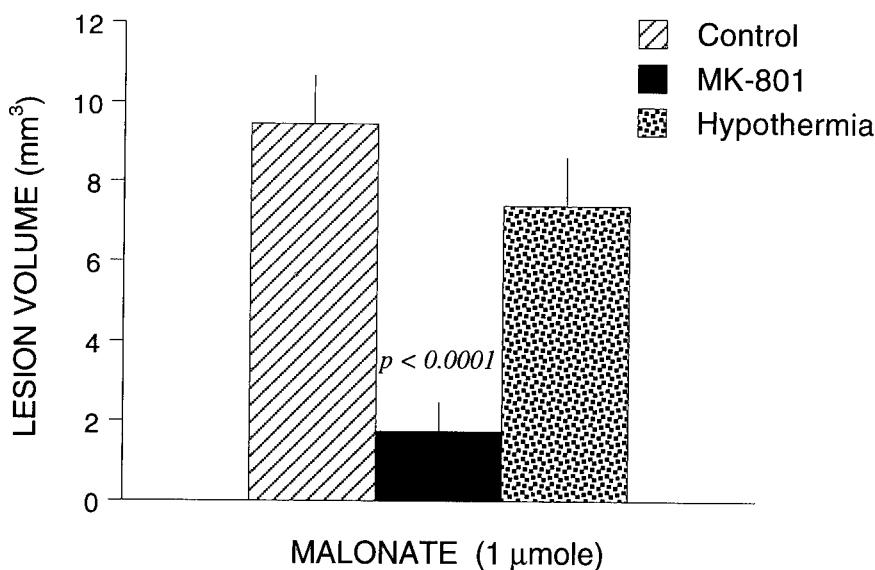


Figure 2 (A) Mean core body temperature over time following surgery in control, MK-801-treated, and anesthesia-induced hypothermic rats. Each point represents mean \pm SEM of 10 animals. MK-801 did not induce hypothermia at any time point. (B) Striatal lesion volume of rats from control (n=16), MK-801-treated (n=13), and anesthesia-induced hypothermic (n=11) rats. MK-801 provided profound neuroprotection, but hypothermia did not. *Reproduced with permission from the Journal of Neurochemistry.*

Figure 2b indicates that even in the absence of any hypothermia, MK-801 is highly neuroprotective. Furthermore, anesthesia-induced hypothermia was not protective against the toxicity of malonate injection (Fig. 2). These results indicate that the protective effect of MK-801 is mediated by its NMDA receptor blockade and not by a nonspecific temperature effect¹⁸. This conclusion is further supported by data indicating that LY274614, a potent competitive NMDA antagonist³², and 7-chlorokynurename, a glycine site antagonist, are both neuroprotective¹⁸.

Several other reports indicate that the neurotoxicity of malonate is mediated by the NMDA receptor^{4,20}. Further implicating the glutamate system, malonate toxicity is prevented by both prior decortication to remove striatal glutamatergic input and inhibition of glutamate release by lamotrigine²⁰. Malonate toxicity appears to be mediated exclusively by the NMDA subtype of glutamate receptor because the non-NMDA antagonist NBQX is not neuroprotective against malonate (Fig. 1)¹⁸.

Co-injection of glutamate receptor agonists with malonate has also proved helpful in defining the relationship between metabolism and excitotoxicity. The general design of these experiments was the combination of subtoxic doses of malonate and agonist. Co-injection of malonate with NMDA produces a marked synergistic toxicity that is blocked completely by MK-801, but unaffected by NBQX¹⁹. We hypothesize that this toxicity is produced by a mechanism similar to that which has been suggested for malonate, i.e., “weak” or “secondary” excitotoxicity. Under conditions of metabolic inhibition, neuronal ATP levels may be insufficient to adequately activate the Na-K ATPase. Reduced activity of this membrane ion pump results in slow depolarization of the neuronal plasma membrane. This depolarization is very important in light of the voltage-dependent properties of the NMDA receptor²⁷. At resting membrane potential, extracellular magnesium prevents ion flux through the NMDA receptor ion channel, even when agonist is present in sufficient concentrations to activate the receptor. However, when a neuron becomes depolarized, the magnesium blockade is relieved and ions can flow through the channel, causing excitotoxic damage. In this way, low-grade metabolic inhibition by malonate may ease the magnesium block of the NMDA receptor ion channel, facilitating receptor activation at low concentrations of NMDA and leading to excitotoxic neuronal death. In addition, metabolic impairment may also make it more difficult for neurons to handle the normally innocuous calcium influx associated with low grade NMDA receptor activation. Furthermore, under conditions of more pronounced metabolic stress, e.g. higher doses of malonate, Na-K ATPase activity is further compromised, thereby depolarizing the neuronal membrane to a greater extent. This may more completely relieve the magnesium blockade of the NMDA receptor and allow endogenous levels of glutamate to activate the NMDA receptor sufficiently to cause excitotoxic neuronal death.

There is substantial evidence to support the assertion that metabolic stress produces brain damage that is mediated by the NMDA receptor. In cultured cerebellar granule cells, inhibition of metabolism by cyanide increases susceptibility to glutamate toxicity, and this toxicity is prevented by NMDA antagonists²⁶. In chick retina, hypoglycemia and sodium cyanide produce NMDA receptor-mediated “excitotoxic” lesions with no detectable increase in extracellular glutamate³⁹; these lesions are mimicked by potassium-induced membrane depolarization⁴⁰. *In vivo*, the toxicity of NMDA is greatly enhanced by inhibition of SDH by the suicide inhibitor 3-nitropropionic acid³⁴. In addition, a variety of metabolic inhibitors including aminoxyacetic acid², MPP⁺^{35,36}, and manganese⁸ all produce toxicity that is NMDA receptor-mediated.

Furthermore, NMDA antagonists have been shown to prevent, to a variable degree, neurotoxicity produced in models of hypoxia/ischemia^{15,23,28,38}.

Co-injection of subtoxic doses of malonate and AMPA produced a large lesion that was not affected by MK-801, but completely attenuated by NBQX¹⁹. This result demonstrates that although the toxicity of malonate alone is predominantly NMDA receptor-mediated, malonate inhibition of metabolism can greatly exacerbate the damage caused by AMPA receptor activation. It is possible that malonate inhibition of SDH causes increased AMPA receptor activation, perhaps by impairing receptor desensitization. It is also possible that malonate exacerbates AMPA toxicity by another mechanism, perhaps by disruption of neuronal ion homeostasis and exchange. This interpretation also has implications for the toxicity of malonate alone. It is possible that the most important effect of metabolic inhibition by malonate is not neuronal depolarization, but a failure of neuronal repolarization, as suggested by Riepe *et al.*²⁹. In other words, malonate may not increase glutamate receptor activation *per se*, but it may make a neuron unable to cope with the ionic disturbances that accompany normal glutamate receptor activation, so that although the amount of depolarization caused by glutamate agonists (including endogenous glutamate) is not enhanced, the repolarization from agonist stimulation is significantly impaired. In this situation, neuronal death may result from accumulation of neuronal calcium caused by $\text{Na}^+/\text{Ca}^{2+}$ exchange and other ionic mechanisms. This mechanism may apply not only to situations involving AMPA receptor activation, but also those involving NMDA receptor activation. It is also conceivable that a combination of increased glutamate receptor activation and impaired neuronal ion exchange is responsible for the toxicity caused by malonate and other metabolic insults.

Malonate exacerbated glutamate toxicity in a manner that was partially attenuated by MK-801, but was insensitive to NBQX. Unexpectedly, the combination of MK-801 and NBQX was no more effective than MK-801 alone against malonate + glutamate toxicity (Fig. 3)¹⁹. These results suggest that in a situation similar to hypoxia/ischemia (metabolic inhibition and high extracellular glutamate concentrations) neurotoxicity is mediated partly by the NMDA receptor, but also by mechanisms apparently unrelated to ionotropic glutamate receptors. The mechanism of the NMDA receptor-mediated portion of the toxicity is likely to be similar to that of malonate alone and malonate + NMDA. Possible explanations for the portion of toxicity not mediated by ionotropic receptors are currently speculative. Metabotropic glutamate receptors may play a role, but the current lack of potent, specific metabotropic antagonists make this hypothesis difficult to test. Other possibilities may include inhibition of cystine uptake by excess glutamate^{24,25} or activation of voltage-dependent sodium channels^{7,40}. In light of the fact that this toxicity may play a large role in the neurodegeneration associated with hypoxia/ischemia, it deserves serious experimental consideration.

We have also performed experiments to assess malonate toxicity in the hippocampus, a brain region exquisitely sensitive to hypoxic/ischemic damage. Hippocampal injection of malonate produces selective toxicity (Fig. 4a). Neurons of the CA1 region are almost completely destroyed, but the dentate gyrus is not affected. Further experiments using specific injection sites indicate that the regional susceptibility profile of malonate toxicity is CA1 > CA3 >> dentate gyrus; the toxicity is prevented by MK-801 (Greenamyre *et al.*, unpublished results). Interestingly, this regional profile is nearly identical to that seen in hypoxia/ischemia³¹, and it correlates

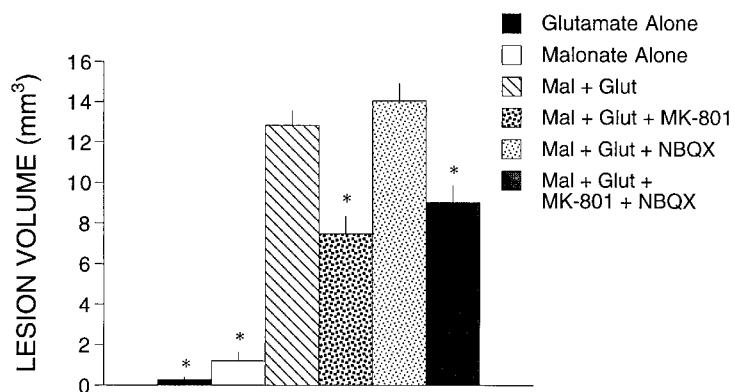


Figure 3 Striatal lesion volume following injection of malonate (Mal) alone, glutamate (Glut) alone, Mal + Glut, Mal + Glut with MK-801 treatment, Mal + Glut + NBQX, or Mal + Glut + NBQX with MK-801 treatment. Each bar represents mean \pm SEM of 5-21 animals. Note the synergistic toxicity of Mal + Glut and the large, but incomplete, neuroprotective effect of MK-801. The (*) denotes significant difference from Mal + Glut ($p < 0.01$, ANOVA and two-tailed Dunnett procedure). *Reproduced with permission from the Journal of Neurochemistry.*

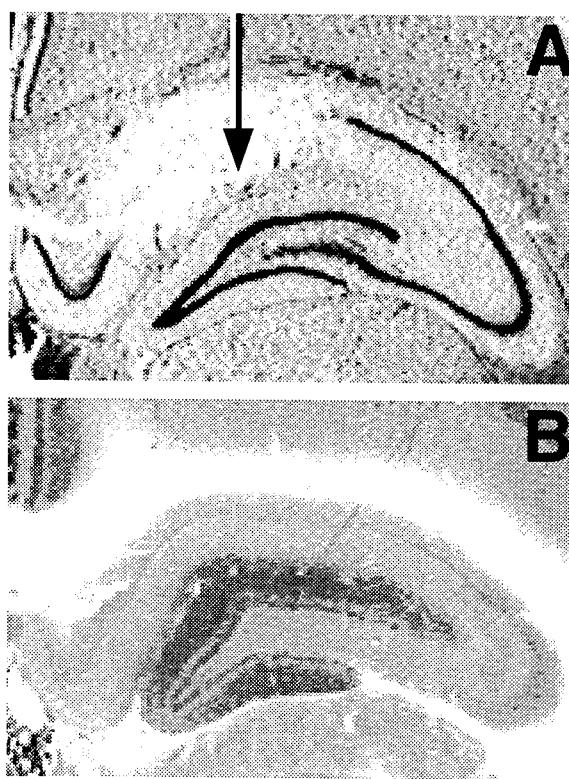


Figure 4 (A) Digitized representative Nissl-stained section of hippocampus following injection of 1 μ mol malonate. Arrow indicates the needle track. Note the selective neurotoxicity in CA1. (B) Digitized section of uninjected hippocampus stained for SDH activity. Note the relatively higher SDH activity in the dentate gyrus than CA3, and the low activity in CA1.

inversely with regional SDH activity, which is highest in dentate, intermediate in CA3, and low in CA1 (Fig. 4b). These results suggest that regional sensitivity to hypoxic/ischemic damage in the hippocampus may be dependent on local metabolic capacity and not on NMDA receptor density, which is similar in both CA1 and dentate gyrus^{16,21}.

In summary, malonate lesions are morphologically and neurochemically similar to those produced by hypoxia/ischemia. As such, malonate injection provides a simple, reproducible model of chemically-induced hypoxia that may be useful in defining both the pathogenesis and therapy of hypoxia and ischemia. Thus far, study of malonate neurotoxicity implicates the NMDA receptor as a key mediator of hypoxic/ischemic neurodegeneration.

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CHAPTER 3

ADAPTATION OF THE BRAIN TO PROLONGED HYPOBARIC HYPOXIA: ALTERATIONS IN THE MICROCIRCULATION AND IN GLUCOSE METABOLISM

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Introduction

Oxygen delivery to the adult mammalian brain is under precise and dynamic control so that the brain is usually exposed to the minimal necessary amount of oxygen. Thus, oxygen may be considered a "necessary evil" whose excess is toxic but whose deficiency has disastrous consequences. Regional brain tissue oxygen tension is not homogeneous; the frequency distribution of local brain oxygen tension is weighted toward lower values, with a mean value that is lower than systemic venous oxygen tension³². In brief and mild hypoxia, regional brain oxygen tension can be maintained by increasing cerebral blood flow¹⁹, which is associated with decreased (faster) capillary mean transit time, increased red cell velocity, and possible capillary recruitment. However, in prolonged hypoxia, several other adaptive changes in systemic physiology and tissue metabolism occur^{8,18,22}. Although these adaptive mechanisms differ by species, the rat's response is similar to man's, and therefore studies of chronic hypoxia in the rat are clinically relevant⁸. Chief among the adaptations (or maladaptations) to prolonged hypoxia are the increased hematocrit and the changes in ventilation and arterial blood gases which increase the oxygen-carrying capacity of the blood. LaManna *et al.* measured the hematocrit, arterial blood gases and cerebral blood flow in rats exposed to hypobaric hypoxia at 0.5 atmosphere (atm) for three weeks and reported that the oxygen delivery to the brain of hypoxic rats was similar to that of controls²⁰. However, tissue oxygen tension in hypoxia remains limited because of the decreased capillary PO₂ which is the driving pressure for oxygen diffusion in tissue. It is presumably for this reason that the brain's vascularity increases in prolonged hypoxia, yielding shorter intercapillary distances, and improved brain oxygenation. The increased brain vascularity in experimental animals subjected to chronic hypoxia has been repeatedly documented^{6,10,12,20,25,28}.

In this review, we summarize recent pertinent results from our laboratories concerning the alterations in the cerebral microvasculature that take place in adult rats subjected to hypobaric hypoxia. Because brain capillaries have an abundance of the glucose transporter (GLUT-1)⁹, and because the brain relies almost entirely on glucose for its oxidative metabolism, we shall also address the effects of the increased brain vascularity on the blood-to-brain glucose transport and brain glucose metabolism. In all experiments we induced hypoxia in adult male Wistar rats (3-6 months of age) by placing them in chambers maintained at 0.5 atm for the indicated times, except for one hour per day when the pressure was returned over 10 minutes to atmospheric for cage cleaning and for water and food replenishment²⁰. Experimental hypoxic rats were always compared to normoxic littermates that were kept outside the hypobaric chamber, but which were otherwise treated in a similar manner.

Architectural Alteration in Cerebral Microvessels After Hypobaric Hypoxia

The hypoxia-induced increased brain vascularity can theoretically be caused by one of three factors: tissue shrinkage, capillary hyperplasia, or capillary hypertrophy. Tissue shrinkage, which is a prominent factor in hypoxic skeletal muscles²⁹ and in severe brain hypoxia⁶, is not an appreciable factor in the mild to moderate hypoxia model that was used here, where total brain weight was decreased by 6% after three weeks of hypobaric hypoxia¹².

It is generally accepted that the brain endothelium is a stable tissue with rare mitoses. Brain capillary growth has been most elaborately studied during development¹ and is believed to rely on two mechanisms: branching or sprouting, and elongation or nonsprouting. Branching is believed to result primarily from mitotic multiplication and longitudinal separation of post-mitotic endothelial cells into sprouts, i.e., hyperplasia. On the other hand, nonsprouting elongation is thought to depend on flattening and hypertrophy of the capillary endothelial cells. Little is known about the mechanisms that underlie the increased brain vascularity of the adult brain in prolonged hypoxia. Specifically, it is not known whether this hypoxia-induced increased vascularity is associated with increased capillary density due to sprouting and branching, or with lengthening of capillary segments and increased tortuosity and loop formation. Previous investigations of brain vascularity in hypoxia employed two-dimensional measurements^{6,10,12,20,25,28}. Because brain capillaries are not always parallel, and can be quite tortuous^{11,27}, estimates of capillary length and geometry in three-dimensional studies are preferable. Mironov *et al.*²⁶ investigated the three-dimensional geometry of the microvascular network in the cerebral cortex of rats exposed to hypobaric hypoxia for three weeks using vascular corrosion casts, a method which was previously applied successfully to studies of brain microvascular architecture in other experimental paradigms^{7,21}. Sections of the casts were studied qualitatively by scanning electronmicroscopy for inspection of the gross density of vessels in various layers of the cerebral cortex, and for changes in the pattern of tortuosity and geometrical organization, and for evidence of sprouting (Fig. 1). The sections were also used to quantitate capillary segment length, i.e. the distance between two capillary branching points, in the same cortical regions. Qualitative inspection in control rats showed the typical cerebral capillary network, with a low vascular density in layers one and two, increasing vascularity in the inner part of layer three and in layers four and five, and decreased vascularity in layer six and in the underlying white matter. In hypoxic rats, the vascular pattern was similar to that of normoxic rats except that

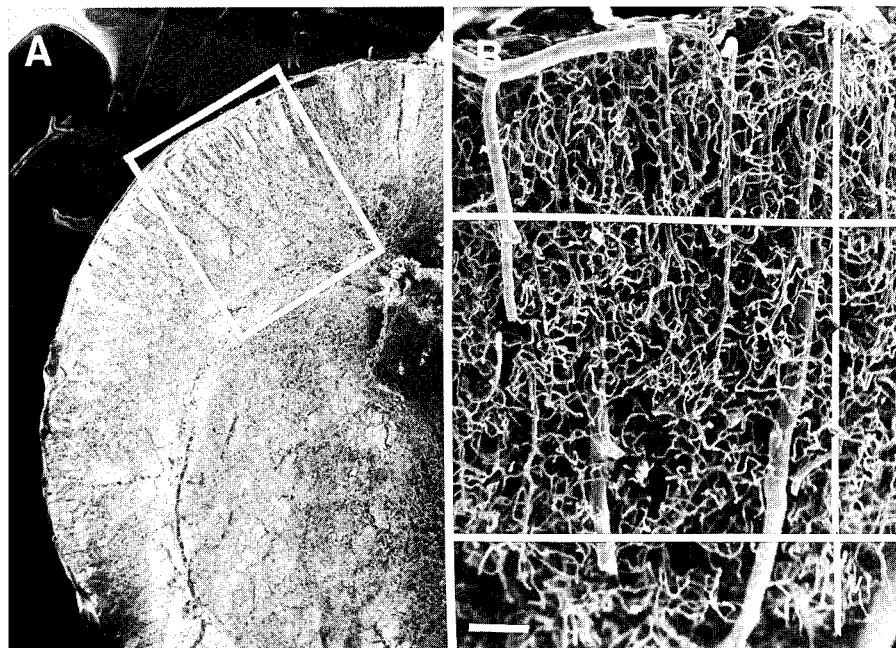


Figure 1 A: low magnification photograph of a coronal section of the vascular cast of a control rat brain about 10 mm anterior to the interaural plane. B: medium magnification photograph of that region of the parietal cortex that is boxed in A. The pial surface is at the top and the cortical layers are identified on the right. The horizontal white lines delineate the superficial (cortical layers 1, 2 and part of 3) and the deep regions of the parietal cerebral cortex that were quantitatively analyzed. Bar = 100 μ m. Taken from ref. 26.

there was increased vascular density, tortuosity and loop-like formation, which was most evident in the deeper part of the cerebral cortex (Fig. 2). There were no capillary sprouts observed in normoxic or hypoxic rats.

The mean capillary segment length was calculated for the superficial and deep layers of the parietal cortex. There was a significant increase in the mean capillary segment length in the deep parietal cortex of hypoxic rats compared to that of normoxic rats. However, significant differences in capillary segment length were not observed between the superficial regions of hypoxic and control rats, nor between the deep and superficial cortex of control rats²⁶. The difference in capillary segment lengths in the deep parietal cortex of normoxic and hypoxic rats was further characterized by comparing the frequency distribution of the capillary segment lengths (Fig. 3). In hypoxia, the peak frequency was increased from $37.1 \pm 1.7 \mu$ m to $52.3 \pm 1.9 \mu$ m (mean \pm SD). If sprouting were the only mechanism for the increased vascularity of the hypoxic cerebral cortex, then a smaller capillary segment length would be predicted. However, the opposite findings suggest capillary elongation and hypertrophy. The qualitative impression of the increased cerebral vascularity in hypoxic rats, particularly in the deeper layers of the cortex (Fig. 2) is consistent with prior reports based on two-dimensional studies of brain sections²⁰.

The failure to observe definite morphological evidence of budding capillaries with cone formation may be related to the time at which the vascular casts were performed. It is possible that by three weeks of hypoxia, the budding process was already com-

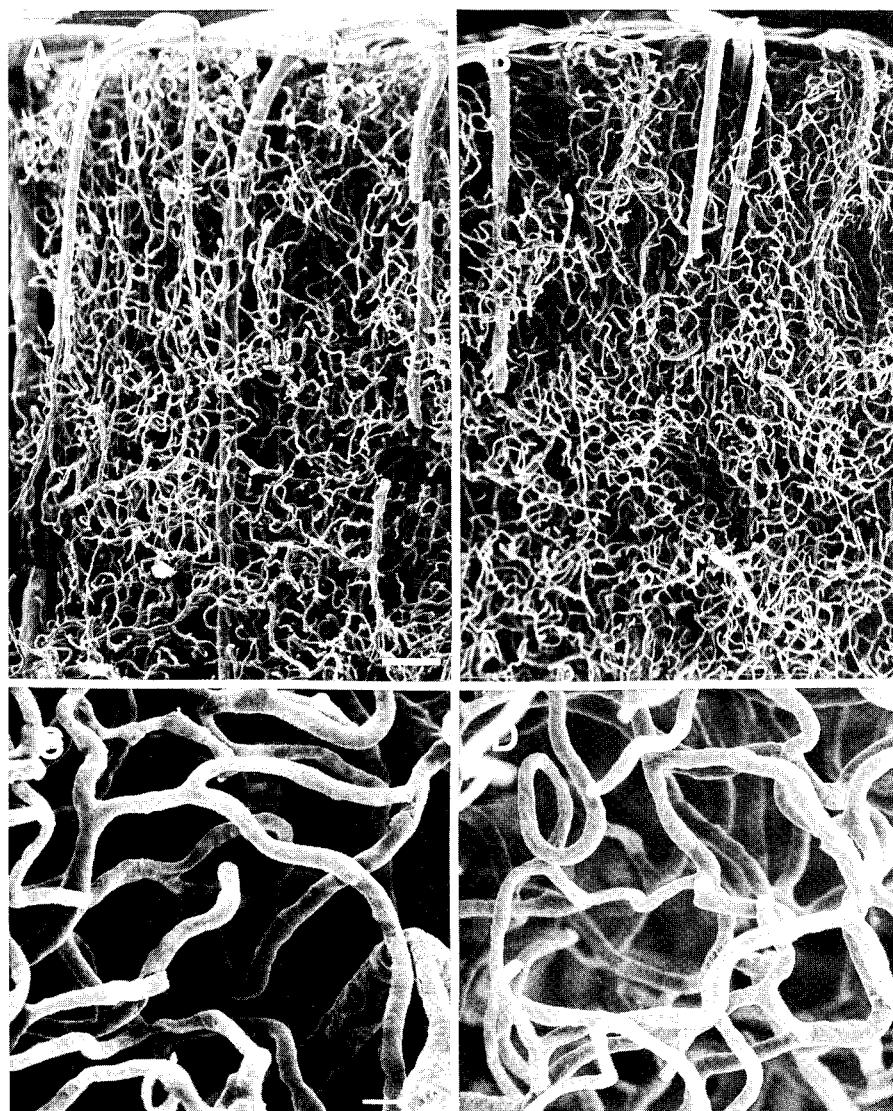


Figure 2 Medium (A and B) and higher (C and D) magnification photographs of vascular casts of the parietal cerebral cortex from a control (A and C) and a hypoxic (B and D) rat. A and B include all cortical layers with the pial surface on top and have the same magnification (bar = 100 μ m). Note the increased vascular density in hypoxia. Taken from ref. 26.

plete. Also, capillary buds may be hard to demonstrate by the corrosive cast technique, particularly if the process of lumen formation is quick. One cannot detect sprouts without lumen, and canalized sprouts cannot be identified if they had already connected to another vessel. For this reason, morphological studies using endothelial markers may shed new light on the mechanisms that underlie the hypoxia-induced alterations in the brain vasculature.

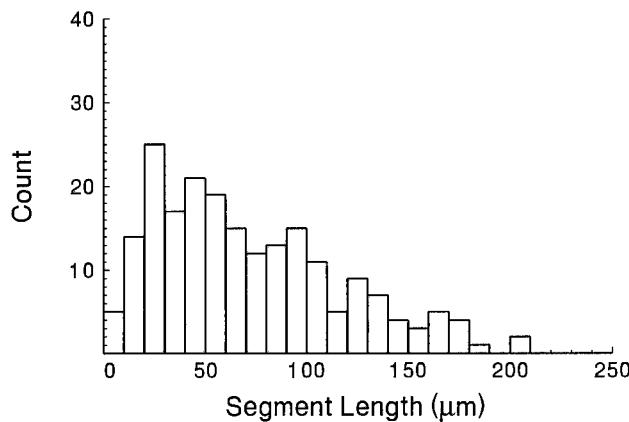
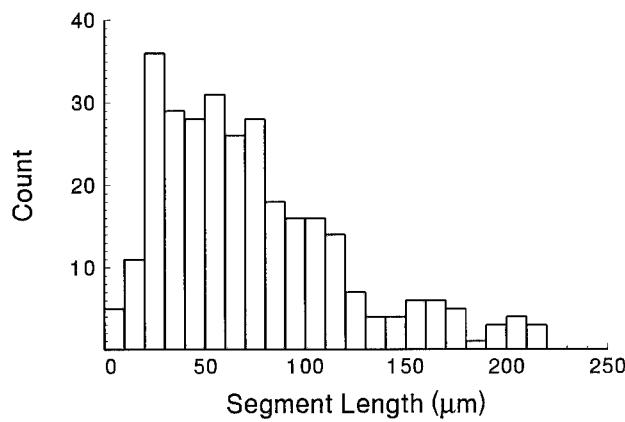
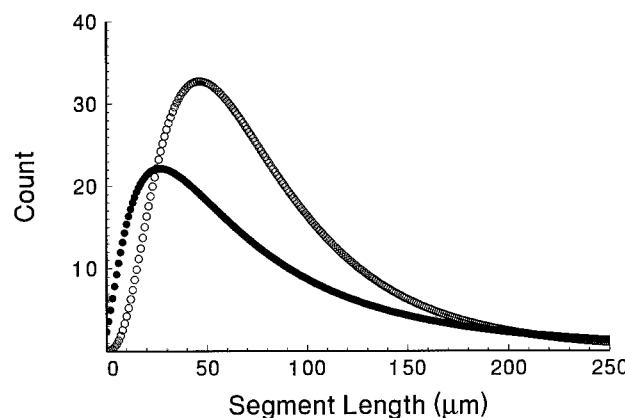
A**B****C**

Figure 3 Frequency distribution of capillary segment lengths in the deep region of the parietal cortex of a normoxic (A) and in a hypoxic (B) rat. The counts in the ordinates indicate the number of segments that were counted. The distribution curves (C, filled circles for normoxia and open circles for hypoxia), were fitted to a log-normal equation ($r^2 = 0.95$ for each) and the peak distributions were significantly different at $P < 0.05$. Taken from ref. 26.

Effect of Hypobaric Hypoxia on the Protein and DNA Content of Cerebral Microvessels

Harik *et al.*¹³ studied the relative contributions of microvascular hypertrophy and hyperplasia to the increased brain vascularity in rats exposed for one, two or three weeks to hypobaric hypoxia. At the end of hypoxic exposure, the rats were decapitated and their cerebral cortical mantles dissected free of meninges, weighed and the microvessels were harvested from the cerebral cortical mantles pooled from 3-6 rats. Cerebral microvessels were obtained by bulk isolation^{12,13}. The isolated cerebral microvessels from normoxic and hypoxic rats were washed and then homogenized in distilled water. Aliquots of the homogenates were assayed for protein and DNA. Cerebral microvessel yield was expressed in mg of microvessel protein per g wet weight of cerebral cortex. Cerebral microvessel DNA yield was expressed in μg of microvessel DNA per g wet weight of cerebral cortex. Cerebral microvessel cell size index was calculated as the ratio of microvessel protein to microvessel DNA (mg/mg).

Isolated cerebral microvessels from control and hypoxic rats appeared similar by interference contrast microscopy. Consistent with previous descriptions, the vast majority of the isolated microvessels were less than 10 μm in diameter, and it was estimated that the microvessels with smooth muscle in their walls (arterioles and venules) constituted less than 15% of the microvessel preparations. Even in this minority of vessels, those with more than one layer of smooth muscle in their walls were rarely encountered. Thus, we believe that the endothelial cells and pericytes constitute the majority of our microvessel preparation, with only minor contribution from

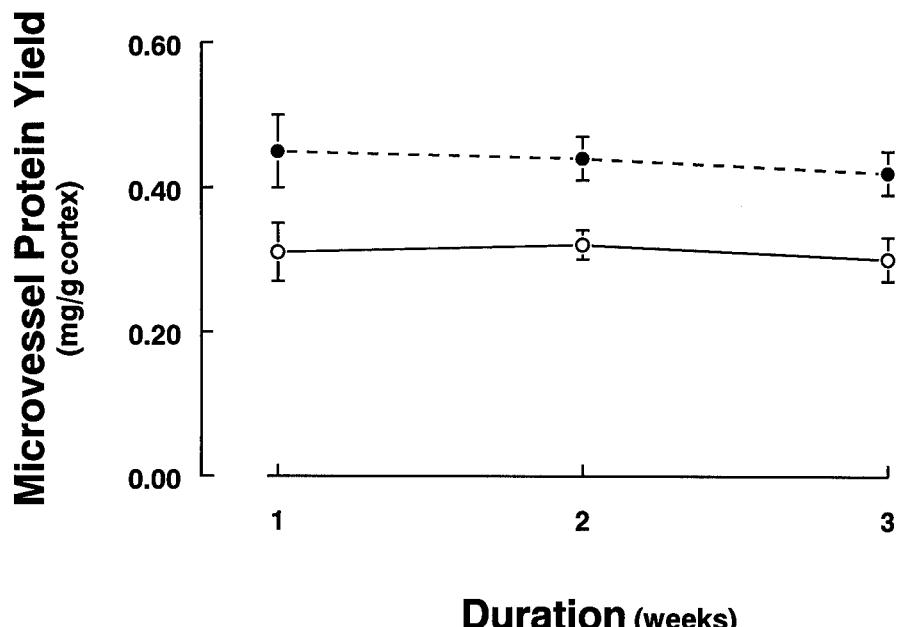


Figure 4 Effects of hypoxia on cerebral microvessel protein yield. Cerebral microvessel protein yield in normoxic (open dots) and hypoxic (closed dots) rats as a function of the experimental duration. The data represent means \pm SE of five experimental observations at weeks one and two, and four observations at week three. Analysis of variance showed an effect of hypoxia ($P<0.001$) but no effect of duration ($P=0.888$). There was also no difference in the hypoxia effect at different weeks ($P=0.866$). Taken from ref. 13.

smooth muscle cells. These findings are consistent with the qualitative observations that the yield of the microvessel pellets obtained from the cortical mantles of hypoxic rats was noticeably larger than that from normoxic rats¹².

The quantitative results showed that cerebral microvessel protein yield in control rats was about 0.31 mg of microvessel protein per g cerebral cortex at all the time periods that were studied (Fig. 4). Assuming no appreciable microvessel loss in the isolation procedure, and that protein constitutes about 10% of the wet weight of the tissue²⁴, this result means that microvessels account for at least 0.3% of the wet weight of the cerebral cortex. The cerebral microvessel protein yield increased to 0.45 mg of microvessel protein per g cerebral cortex at one week of hypoxia (about a 45% increase), and did not increase further with longer hypoxic periods (Fig. 4).

Cerebral microvessel DNA yield in normoxic rats was about 35 µg of microvessel DNA per g cortex. There were no significant differences among the three time points in normoxic rats (Fig. 5). Assuming that rat diploid cells, like human cells, contain 7 pg of DNA per cell, this finding translates to about 5 million microvascular cells per g cerebral cortex. Despite the rise in cerebral microvessel protein yield at one week of hypoxia, there was no increase in cerebral microvessel DNA yield, indicating lack of mitosis in cerebral microvessels at that time period. However, there were significant increases in the yield of cerebral microvessel DNA over normoxic controls by two and three weeks of hypoxia (Fig. 5). The calculated results for the microvascular cell

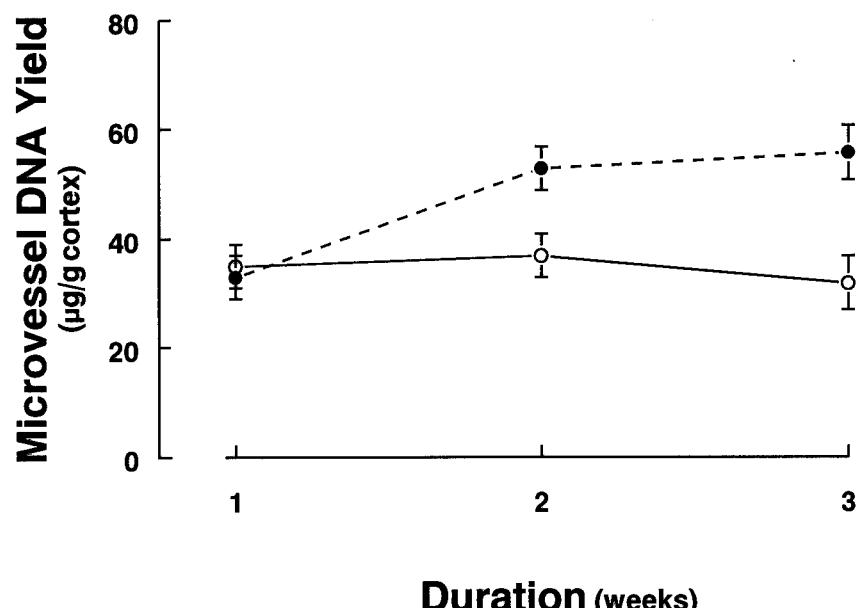


Figure 5 Effects of hypoxia on cerebral microvessel DNA yield. Cerebral microvessel DNA yield in normoxic (open dots) and hypoxic (closed dots) rats as a function of the experimental duration. The data represent means \pm SE of five observations at weeks one and two, and four observations at week three. Analysis of variance showed no significant differences among normoxic rats at the three time points, but there were significant differences among the hypoxic groups: weeks two and three were not different from one another ($P=0.331$), but both were greater than week one ($P<0.001$). Consequently, the hypoxia effect was significant at weeks two and three but not at week one. Taken from ref. 13.

size index are depicted in Figure 6. The microvessel cell size index increased from 8.8 to 13.9 at one week of hypoxia, but then decreased to normoxic values at two weeks of hypoxia and continued to decrease to a mean significantly below normoxia by three weeks of hypoxia.

These findings confirm prior impressions that hypoxia increases the brain vascularity. Cerebral microvessel protein yield increased within one week of hypoxia but did not increase further with prolonged hypoxia. In contrast, cerebral DNA yield did not change by one week of hypoxia indicating that the increased brain vascularity at one week was due to microvascular hypertrophy. With continued hypoxia, there was a significant increase in cerebral microvessel DNA yield which eventually exceeded the increased protein yield, implying hyperplasia as the later mechanism underlying the increased vascularity of the brain in persistent hypoxia. It was noted that the isolated microvessel preparation consists mostly of endothelial cells and pericytes with a minority of smooth muscles, and it was presumed that the reported hyperplasia was caused by mitosis primarily in the endothelium given the suspected anti-proliferative role of pericytes¹. The ratio of endothelial cells to pericytes in brain capillaries in hypoxia remains to be investigated.

Hypoxia-induced changes in brain vascularity demonstrate the capacity for structural plasticity in the adult rat brain. These changes are probably one aspect of the general phenomenon whereby capillary density is matched to the energy requirements of the brain. It is known that regional brain capillary density is proportional to

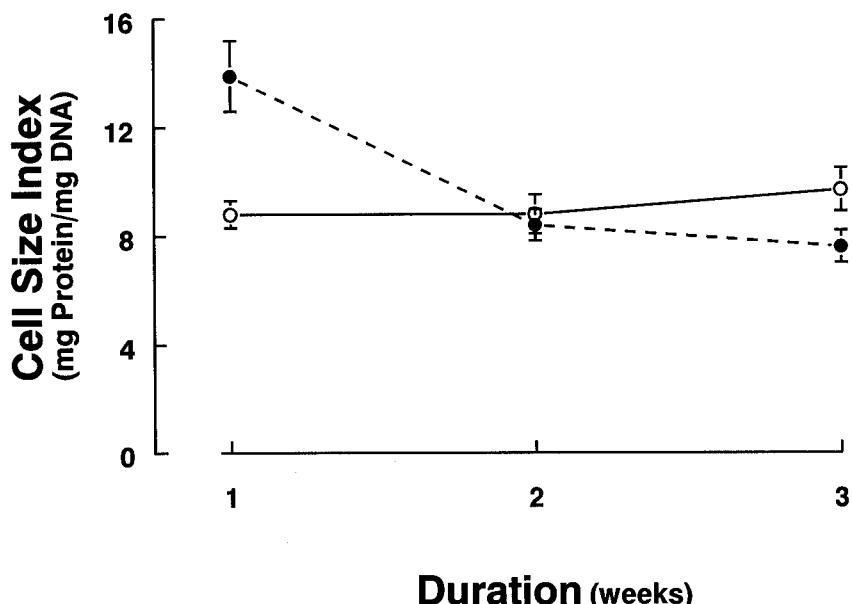


Figure 6 Effects of hypoxia on cerebral microvessel cell size. Cerebral microvessel cell size index in normoxic (open dots) and hypoxic (closed dots) rats as a function of the experimental duration. The data represent means \pm SE of five observations at weeks one and two and four observations at week three. Analysis of variance showed no significant differences among normoxic rats at the three time points, but there were significant differences among the hypoxic groups: week one differs from weeks two and three. There were no differences between weeks two and three. The hypoxia effect was significantly positive at week one ($P<0.001$), not significant at week two ($P=0.607$), and significantly negative at week three ($P<0.05$). Taken from ref. 13.

regional metabolic rate^{4,17}. It was also shown in adult rats that when the pattern of regional neuronal activity was altered by environmental enrichment³ or by forced exercise and motor task learning¹⁶, then brain capillary density was altered to reflect the new regional pattern of brain activity.

Hypobaric Hypoxia Increases Glucose Transport at The Blood-Brain Barrier

Brain capillaries are known to have an unusually high density of the glucose transporter protein (GLUT-1) in their plasma membranes⁹. This is understandable since brain capillaries, which constitute less than 1% of the wet weight of brain, have to transport glucose for the overwhelming mass of surrounding neurons and glia. Because oxygen, but not glucose is limited in hypobaric hypoxia, we initially suspected that newly formed brain capillaries in hypobaric hypoxia would have a lower GLUT-1 density in their plasma membranes to avoid a mismatch between oxygen and glucose delivery.

To address this question, Harik *et al.* quantitated GLUT-1 in isolated cerebral microvessels pooled from rats that were subjected to hypobaric hypoxia for one or three weeks, and in their littermate controls¹². The isolated cerebral microvessels were assayed for their D-glucose-displaceable [³H]cytochalasin B (CB) binding. Also, specific CB binding was performed on particulate fractions of samples of the cerebral cortex of these rats. Specific CB binding was determined at several concentrations of the ligand, and the maximal binding (B_{max}) and the dissociation constant (K_d) were determined. Given that GLUT-1 is the predominant form of GLUT in cerebral microvessels, the B_{max} of CB in isolated cerebral microvessels is believed to portray the density of GLUT-1 in this tissue. Specific CB binding to cerebral microvessels was saturable¹². Scatchard analyses³¹ of the results from hypoxic and control rats yielded linear plots, indicating a single class of binding sites. The B_{max} was significantly increased by about 25% at both one and three weeks of hypoxia, but the dissociation constant values were not altered (Table 1). CB binding to particulate fractions of the cerebral cortex was not affected by hypoxia¹².

Because CB binding sites are several-fold higher in brain microvessels than in brain tissue, the purity of brain microvessels from hypoxic and control rats was assessed by assaying their γ -glutamyl transpeptidase activities in addition to microscopic inspection. Microscopic evaluation of isolated microvessels showed no significant differences between those obtained from hypoxic and normoxic rats. However, γ -glutamyl transpeptidase activity, which is known to be 20- to 30-fold higher in cerebral microvessels than in the cerebral cortex from which the microvessels were obtained⁹, was significantly decreased in cerebral microvessels after one and three weeks of hypobaric hypoxia (Table 1). These results suggest either that γ -glutamyl transpeptidase activity in isolated cerebral microvessels is decreased in hypoxia, or that brain microvessel preparations from hypoxic rats are less pure. The latter possibility should have resulted in lower, rather than higher, CB binding to hypoxic brain microvessels¹².

To determine whether the combination of increased vascularity of the brain and the increased density of GLUT-1 in cerebral microvessels of rats subjected to hypobaric hypoxia is associated with increased blood-to-brain glucose transport, Harik *et al.* measured the brain influx of glucose *in vivo* in rats subjected to three weeks of the same hypobaric insult, and in control rats¹². Regional blood-to-brain D-glucose transport and blood flow were determined in some rats, and the regional L-glucose space

Table 1. Effect of hypobaric hypoxia on CB binding and GGT activity in isolated brain microvessels

	CB Binding		
	Bmax pmol/mg protein	K _d μM	GGT Activity μmol.mg protein ⁻¹ .h ⁻¹
1 Week hypoxia			
Control	56.7±6.6	0.27±0.11	11.92±1.70
Hypoxia	69.2±8.8	0.26±0.13	9.79±1.47
P value	<0.005	NS	<0.005
3 Weeks hypoxia			
Control	54.7±15.4	0.22±0.14	11.60±2.0
Hypoxia	70.6±16.5	0.21±0.09	8.69±1.0
P value	<0.001	NS	<0.001

Values are means \pm SD; n = 4 and 7 paired experimental observations at 1 and 3 wks of hypoxia, respectively. CB, cytochalasin B; GGT, γ -glutamyltranspeptidase; Bmax, maximal binding; K_d, dissociation constant. Significant differences were calculated by paired Student's t-test (2-tailed). Taken from ref. 12.

and blood flow were determined in other rats by the double-labeled single-pass indicator-fractionation atrial bolus injection method¹². The data allowed measurements of regional cerebral blood flow, the volume of distribution of L-glucose, and the extraction fraction of D-glucose. The data also allowed calculation of unidirectional blood-to-brain glucose influx and the maximal blood-to-brain glucose transport capacity values.

Cerebral blood flow was slightly higher in hypoxic rats, but the difference did not reach significance in any of the brain regions, which is compatible with the results of LaManna *et al.*²⁰. Also, there were no significant alterations in regional brain L-glucose space in hypoxic rats¹², which is consistent with the reported lack of alteration in regional brain sucrose space in hypoxia²⁰. The extraction fraction values of D-glucose were increased by about three-fold in hypoxic rats (Table 2). The unidirectional blood-

Table 2. Effect of hypobaric hypoxia on brain D-glucose uptake

Brain Region	Extraction Fraction		Glucose Influx, μmol.100g ⁻¹ .min ⁻¹		Tmax, μmol.100g ⁻¹ .min ⁻¹	
	Control	Hypoxia	Control	Hypoxia	Control	Hypoxia
Frontal cortex	0.25 ± 0.08	0.72 ± 0.27*	212 ± 86	382 ± 118*	287 ± 104	522 ± 147*
Parietal cortex	0.24 ± 0.08	0.65 ± 0.25*	221 ± 84	407 ± 127*	307 ± 118	569 ± 174*
Hippocampus	0.28 ± 0.10	0.76 ± 0.34*	173 ± 68	294 ± 83*	208 ± 82	354 ± 93*
Striatum	0.27 ± 0.10	0.75 ± 0.27*	204 ± 94	290 ± 123	222 ± 102	316 ± 132
Cerebellum	0.32 ± 0.12	0.73 ± 0.34*	214 ± 98	300 ± 135	276 ± 126	391 ± 169

Values are means \pm SD; n = 4 control and 6 hypoxic rats. Hypoxic rats were kept in hypobaric chamber for 3 wks. Tmax, maximal blood-to-brain transport capacity. *Significant difference between 2 groups by 2-sample Student's t-test (2-tailed) at P < 0.05. Taken from ref. 12.

to-brain glucose influx was doubled in hypoxic rats (Table 2). That the increased brain glucose influx in hypoxia was not commensurate with the increased extraction fraction despite the similar regional blood flow and the similar L-glucose space in both groups is probably a reflection of the markedly decreased blood plasma fraction which is a consequence of the increased hematocrit and the decreased blood plasma fraction in hypoxic rats. The calculated maximal transport capacity was significantly increased in hypoxic rats¹².

Thus, hypoxia not only increases the density of GLUT-1 in isolated cerebral microvessels, but it also induces a major increase in the blood-to-brain glucose transport. The increased GLUT-1 density in isolated cerebral microvessels from rats subjected to hypoxia is compatible with the observation that hypoxia increases the expression of GLUT-1 mRNA and protein in cultured bovine aortic endothelial cells *in vitro*²³, and with the report that hypobaric hypoxia increased the levels of GLUT-1 mRNA and protein but decreases the levels of GLUT-4 mRNA and protein in the rat heart³³. However, it should be cautioned that the increased density of GLUT-1 is not necessarily synonymous with increased glucose transport. GLUT-1 density is a static value, whereas glucose transport is a dynamic rate. Nonetheless, Harik *et al.* directly demonstrated an increased blood-to-brain glucose transport *in vivo* commensurate with the increased CB binding in hypoxic brain microvessels and with the increased microvessels density in hypoxic brains¹². Thus, in the cerebral cortex where the capillary density increased by about 60%²⁰ and GLUT-1 density increased by 30%¹², they measured an 80% increase in glucose influx (Table 2). Therefore adaptation to hypoxia in the rat results in near doubling of the blood-to-brain glucose transport as a result of both the increased brain vascularity and the increased GLUT-1 density per mg of microvessel protein.

Harik *et al.* hypothesized that the higher GLUT-1 density in brain microvessels of rats subjected to prolonged hypobaric hypoxia occurred in response to the decreased brain plasma flow rate¹². In hypoxic adapted rats, oxygen delivery is maintained or slightly elevated mainly because of the increased hematocrit, whereas brain blood flow values return to normal²⁰. This means that the brain plasma flow rate is decreased by about 50%. Because in the rat, glucose is available only from the plasma, the glucose extraction fraction would have to double just to maintain a constant brain glucose influx. A higher brain glucose influx might be designed to bring more glucose into the brain even in the absence of appropriate amounts of oxygen for possible increased glycolytic metabolism.

Effects of Hypobaric Hypoxia on Brain Glucose Metabolism

Acute mild hypoxia causes increased brain glucose consumption^{2,30}. To determine whether prolonged hypobaric hypoxia is associated with altered brain glucose metabolism, Harik *et al.* measured regional brain concentrations of glucose, glycogen, lactate, adenosine triphosphate (ATP), phosphocreatine (PC), regional intracellular pH, and regional cerebral metabolic rate for glucose (CMR_{glc}) in rats subjected to three weeks of hypobaric hypoxia¹⁴. For the study of brain metabolites and pH, the hypoxic and normoxic rats underwent *in situ* brain freezing. CMR_{glc} was determined by the 2-deoxyglucose autoradiographic method. Results obtained from whole sections of the forebrain indicate a significant increase in brain glucose and lactate levels and a significant decrease in brain glycogen levels but without a significant change in brain ATP or PC levels. Also, there was no difference in brain intracellular pH be-

tween hypoxic and normoxic rats. Thus, the increased glucose transporter at the blood-brain barrier and the increased blood-to-brain glucose transport result in higher brain glucose concentrations. The lack of discrepancy between hypoxic and normoxic brains in their ATP and PC levels further indicates that at this level of hypoxia, there is adequate compensation for the oxygen deficiency. The similar regional brain intracellular pH in hypoxia and normoxia is another indication of the compensation which prevents tissue acidosis in the face of higher lactate concentrations in the brains of hypoxic rats. Harik *et al.* speculated that the increased brain lactate levels in hypoxia are useful for maintaining brain pH in the presence of low brain tissue CO₂ tension, thereby avoiding the necessity to export brain bicarbonate¹⁴. The concomitant finding of decreased brain glycogen and increased brain lactate suggests increased glycolysis in the hypoxic brain.

The more important finding however is the increased CMRglc in hypoxic rats. It is suggested that the increased CMRglc in hypoxia, combined with the low brain plasma flow in hypoxic rats and the fact that rat erythrocytes do not carry glucose, are logical explanations for the increased glucose transport at the blood-brain barrier¹². The exact mechanism of how the higher CMRglc in prolonged hypoxia causes up-regulation of glucose transport at the blood-brain barrier remains unknown. Near doubling of cerebral lactate and the decreased glycogen levels are highly suggestive of increased glycolysis in hypoxia, which of course is detected by the 2-deoxyglucose method.

This increased CMRglc is probably another compensatory mechanism that underlies adaptation to prolonged hypoxia which we now document in the brain and which was previously suspected in the heart¹⁵. Perhaps this is the reason why a carbohydrate diet is believed to be beneficial for subjects exposed to high altitude⁵.

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CHAPTER 4

HEMOGLOBIN ADAPTATIONS TO HYPOXIA AND ALTITUDE— THE PHYLOGENETIC PERSPECTIVE

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Introduction

Some species of endothermic vertebrates exhibit exceptional tolerance to altitudinal hypoxia. Generally, however, birds are more tolerant than mammals as judged from the altitudes tolerated and number of species that live and breed at high altitude⁵. The bar-headed goose that breeds in south-central Asia and overwinters on the Indian subcontinent tops Mt Everest at approximately 9 km height³⁹ and Ruppel's griffon soars over Western Africa at 11.3 km²⁰, where the ambient O₂ tension (36 torr) is less than a quarter of that at sea level. The problem of securing adequate uptake and internal transport of O₂ is compounded by a sharp increase in tissue energy demand during flight (a 15 fold increase compared to resting values at sea level)⁹.

Maintenance of tissue O₂ supply under extreme conditions depends on a symphony of organismic, cellular and molecular adaptations. Compared to the sac-like alveolar lung of tetrapod vertebrates, the unique functional anatomy of the avian lung correlates with greater gas exchange efficiency as is evident from high ventilatory minute volumes and arterial PO₂ values that may exceed those in expired air. The adaptations evident at the organismic and cellular levels of biological organization have been subject to recent review in the physiological literature^{21,38}.

This treatise is centered on phylogenetic adaptations manifested in hemoglobins (Hbs) from adult birds and mammals that experience pronounced ambient hypoxia and Hbs from fetal and embryonic stages of mammals that lack direct access to atmospheric air and exploit homologous molecular strategies. Focusing on the bar-headed goose it illustrates how adaptations involving single amino acid substitutions in the Hb molecule may play a governing role in determining tolerance to extreme altitudes.

A: Molecular Basis for Functional Adaptations

Vertebrate Hb molecules are tetrameric each binding four O₂ molecules. Adult Hbs ($\alpha_2\beta_2$) consist of two α and two β protein chains (Fig. 1) that in humans are composed of 141 and 146 amino acid residues, respectively. Before birth some mammals have fetal Hb (HbF that has γ instead of β chains) and in earlier developmental stages all mammals appear to express embryonic Hbs that contain embryonic α or β -

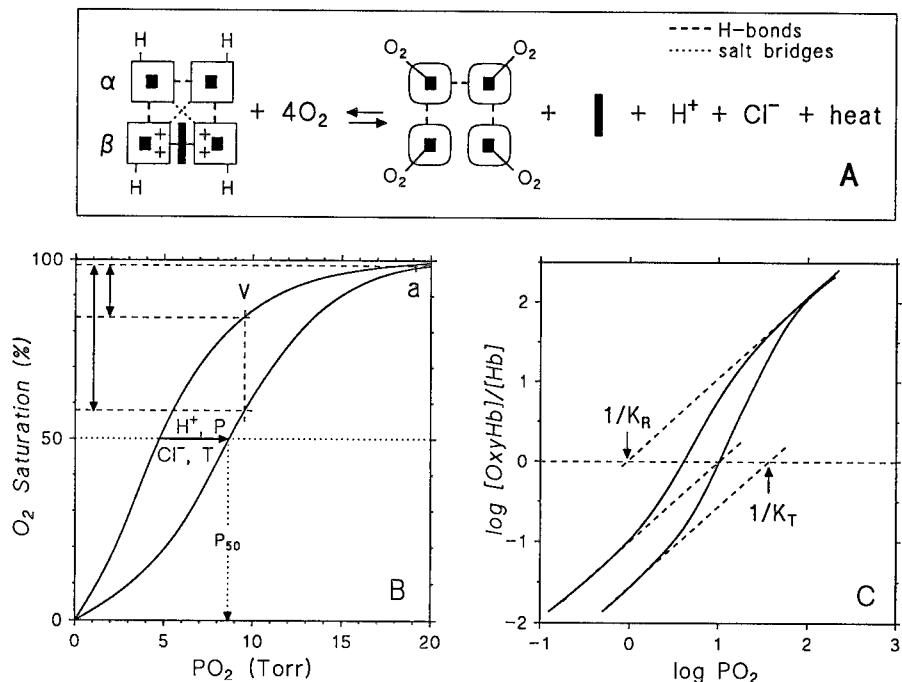


Figure 1 A, Diagram of the oxygenation reaction of Hb illustrating breakage of salt bridges and the shift from the T(ense) to the R(elaxed) states with liberation of protons, organic phosphates (like DPG), indicated by solid bar, chloride ions and heat. B, O₂ equilibria curves showing decreased O₂ affinity (increased half-saturation O₂ tension, P₅₀) with increases in temperature and phosphate, chloride and proton concentrations. C, Hill plots showing a decreased O₂ affinity of the T-state induced by these effectors.

type chains (Fig. 4). The chains are composed of helical segments joined by non-helical 'corners'. In the deoxygenated state the subunits are braced in a T(ense) conformation by salt bridges that are broken upon oxygenation allowing transition to the (R)elaxed conformational state (Fig. 1). Effectors modulate O₂ affinity by strengthening or weakening the R or T states. Thus the Bohr effect (decreased O₂ affinity when pH falls, which liberates O₂ in the relatively acid tissues) results from proton and CO₂ binding that reduces O₂ affinity by strengthening the T state (Fig. 1). Organic phosphates, like 2,3-diphosphoglycerate (DPG) found in mammalian and inositol pentaphosphate (IPP) found in avian red cells, and chloride ions decrease O₂ affinity by homologous interaction²⁷⁻⁴¹. Cooperativity between the hemes [that is reflected by the slopes of Hill plots (Fig. 1C)] increases the capacitance of blood (O₂ released for a given fall in PO₂) and results from concerted transitions between the T and R states.

All subunits display sequence homology that reflects gene duplication during phylogeny. Molecular adaptations involve mutations at only a few amino acid residues at key positions in the protein moiety. Accordingly most substitutions observed in Hbs of different species are conservative replacements (of internal non-polar residues and external polar and nonpolar ones) that have little effect on functional properties²⁷.

The oxygenation properties of blood depend on (a) the intrinsic O₂ binding properties of the Hb (when 'stripped' of cofactors) and the sensitivity to heterotropic ef-

factors, which are dependent on molecular structure, and (b) the operating conditions in the red cells (e.g. the concentrations of free effector molecules). Whereas interspecific adaptations (that have become encoded in the genes through species differentiation) commonly involve differences in molecular structure of the Hb, intraspecific adaptations (short-term changes occurring within individual animals) commonly involve changes in cofactor concentration. Potentially O_2 transport may be augmented by either an increased O_2 affinity that raises pulmonary loading or a decreased affinity that increases peripheral unloading. Which of these alternatives is applicable depends on the factual arterial and venous *in vivo* O_2 tensions and pH conditions and the arterio-venous O_2 content difference.

As molecular adaptations commonly involve changes in effector binding, the few sites directly implicated need to be reviewed. In human HbA, DPG binds electrostatically at 7 positively charged β chain residues, viz., at the N-terminal valine residues ($\beta 1$ -Val), $\beta 2$ -His and $\beta 143$ -His of both β chains, and at $\beta 82$ -Lys of one chain. The Bohr protons bind predominantly at the N-terminal residues of the α chains and the C-termini of the β chains ($\alpha 1$ -Val and $\beta 146$ -His) and chloride ions at $\alpha 1$ -Val (which interacts with $\alpha 131$ -Ser) and at $\beta 82$ -Lys (which interacts with $\beta 1$ -Val) (Fig. 3). In bird Hbs, IPP appears to bind at the same sites as does DPG in humans, as well as at $\beta 135$ -Arg and $\beta 139$ -His^{27,41}. CO_2 binds at the free N-terminal amino groups of α and β chains.

B: Allosteric Effectors

Within the framework set by molecular structure, allosteric interactions (e.g. with erythrocytic organic phosphates) determine the *in vivo* oxygenation properties of blood.

1. Altitudinal Hypoxia

As illustrated in humans²¹ (Fig. 2), the main adaptive changes in mammals exposed to altitudinal hypoxia ensue from changes in red cell DPG and pH levels, with smaller contributions from alterations in red cell volume and the concentrations of ATP and Cl^- , and of free Mg^{2+} ions that complex with organic phosphates. At moderate altitudes (where O_2 loading is not seriously biased) DPG levels increase, which shifts the blood O_2 equilibrium curve to the right and increases O_2 unloading. This response, which is manifested within a few hours, appears to be mediated mainly by hyperventilation-induced pH increase that affects enzymes implicated in regulating DPG levels. At higher altitudes increasing alkalosis shifts the curve back to the left via the Bohr effect. Thus in humans the *in vivo* O_2 affinity at 4560m approximates that at sea level and becomes even higher at greater altitudes²¹ where a high affinity favours loading. Increasing blood O_2 affinity in rats (by Hb carbamoylation) permits survival at extreme altitude (pressure equivalent to 9180m) where controls die⁸. The lower pH in the tissues compared to the lungs (as evident from lower venous than arterial values) increases capacitance and favours tissue unloading.

Interestingly, second generation hypoxic rats (that experienced hypoxia *in utero*) show an opposite response, compared to first generation ones, to moderate hypoxia (10% O_2), i.e. increased affinity and decreased DPG compared to normoxic controls³³, suggesting that cellular adaptation processes are initiated before birth.

In general the negative correlation between blood O_2 affinity and body weight in mammals^{32,36} appears ascribable to variation in phosphate interaction²⁴, which thus may contribute to a greater hypoxic tolerance in large species.

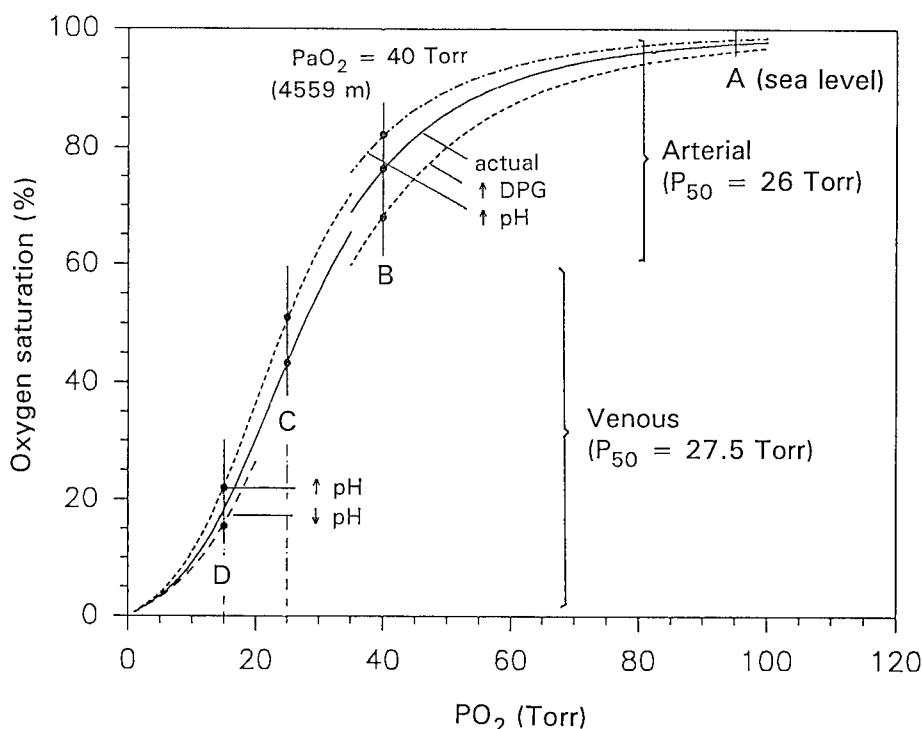


Figure 2 Diagram illustrating effects of increased DPG concentration and pH on arterial and venous O_2 saturations in humans at 4559m altitude, with arterial PO_2 values of 95 and 40 torr (as found at sea level and 4559 m; lines A and B, respectively) and venous values of 25 and 15 torr (lines C and D, respectively). Solid curves reflect actual values, broken curves show values at increased DPG concentration with unchanged pH (\uparrow DPG), at increased pH with unchanged DPG (\uparrow pH) and at decreased pH resulting from high metabolic acidification (\downarrow pH). Modified after ref. 21.

Very little information exists on adaptive changes in cofactor concentrations in birds. In domestic pigeons the effects of the pronounced (39%) decrease in red cell IPP concentration following three weeks' acclimation to (4000m) hypobaria on blood O_2 affinity appears to be cancelled by a concomitant (24%) increase in Hb content³¹.

2. Gestational Development

Fetuses and embryos of viviparous animals generally exhibit higher blood O_2 affinities and lower Bohr effects than adults, which facilitate the transfer of O_2 from the maternal circulation at low O_2 tensions and pH⁴². These stages may express specific embryonic and fetal Hb components with different subunit composition compared to adult HbA (Fig 4). The affinity difference results from three main adaptive strategies (Fig. 5):

(1.) lower polyphosphate levels in the prenatal than in adult red cells, as occurs in fetal dogs, rats, seals and pigs. This strategy is neatly characterised in the Weddell seal, where decreasing blood- O_2 affinity after birth correlates with increased DPG levels and the stripped fetal and adult hemolysates exhibit the same O_2 affinities, Bohr effects and Hb multiplicity (number and relative concentrations of iso-Hb components)³⁴.

α -TYPE CHAINS

	H Cl ^a					
	\ /					
Human HbA	Val	Leu	Ser	-----	Ala	Ser --
	<i>1</i>	<i>2</i>	<i>3</i>		<i>130</i>	<i>131</i>
α vicuna	Val	Leu	Ser	-----	Thr	Asn --
ζ pig	<i>ac-</i>	Ser	Leu	Thr	Thr	Ile --
ζ mouse	<i>ac-</i>	Ser	Leu	Met	Ser	Thr --
ζ man	<i>ac-</i>	Ser	Leu	Thr	Ser	Val --
						Tyr - Arg
						<i>140</i> <i>141</i>

 β -TYPE CHAINS

	D ²	D ² H	D H Cl ^b	D ² H	H
	I	\ /	\ \ /	\ /	I
β human HbA	Val	His	Leu	Lys	His
	<i>1</i>	<i>2</i>	<i>3</i>	<i>82</i>	<i>143</i>
<i>Mammals; HbA</i>					
β llama/alpaca	Val	Asn	Leu	Lys	His
β elephant	Val	Asn	Leu	Lys	His
β sheep	...	Met	Leu	Lys	His
β cow/goat	...	Met	Leu	Lys	His
β yak	...	Met	Leu	Lys	His
β Cat HbA	Gly	Phe	Leu	Lys	His
β Cat HbB	<i>ac-</i>	Ser	Phe	Lys	His
<i>Fetal and embryonic Hbs</i>					
γ human (HbF)	<i>ac-</i>	Gly	His	Leu	Lys
γ sheep (HbF)	...	Met	Leu	Lys	His
ϵ pig, human; θ pig	Val	His	Phe	Lys	His
ϵ mouse	Val	Asn	Phe	Lys	His
<i>Bird HbA</i>					
β chicken/geese ^d	Val	His	Trp	Lys	Arg
β ostrich/swift ^e	Val	Gln	Trp	Lys	Arg
					Lys
					Tyr - His
					Tyr - His

^aBold print signifies residues expected to influence blood O₂ affinity;

^bphosphate binding at both β -chains; ^aalso interacts with α 131-serine;

^balso interacts with β 1-valine; ^cpartly acetylated; ^dincludes bar-headed,

Andean and greylag geese; ^esame as in rhea, starling and blackbird.

Figure 3 Major sites for DPG (D), Bohr proton (H), and chloride (Cl) binding in human HbA and corresponding sites in other Hbs. (Data from refs. 19, 25, 42).

(2.) specific prenatal Hbs with a higher intrinsic O₂ affinity, as exemplified by mammalian embryonic Hbs, and the fetal Hbs from sheep and goats that are insensitive to DPG⁴, and

(3.) lower phosphate sensitivity in fetal than in adult Hb, as observed in humans, where HbF has a lower intrinsic O₂ affinity than HbA, but a higher affinity in the presence of DPG⁴⁰.

The presence of HbF in anemic and hypoxic adult mammals (see below) suggests that its synthesis is not solely under endogenous control but influenced by *in vivo* PO₂.

C. Molecular Mechanisms

1. Intraspecific adaptations

A mounting body of evidence indicates that *in vivo* O₂ tensions may influence the type of Hb synthesized in fetal as well as adult stages, possibly by the same mechanisms.

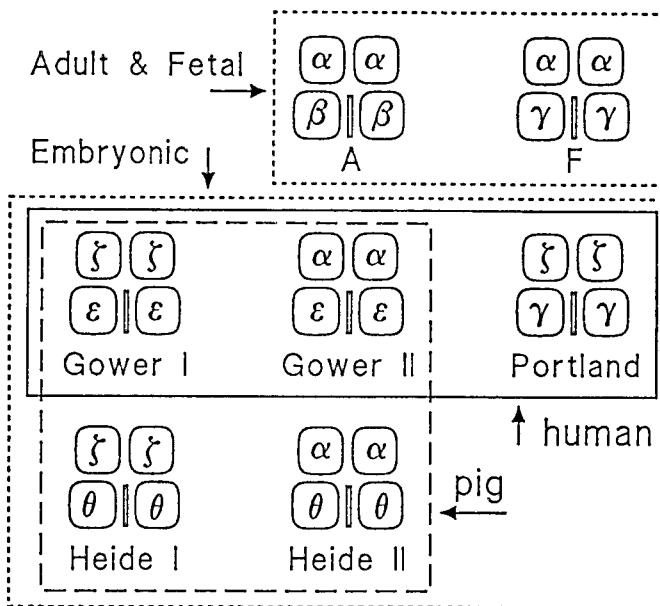


Figure 4 Subunit compositions of human adult and fetal Hbs (A and F) and human and pig embryonic Hbs.

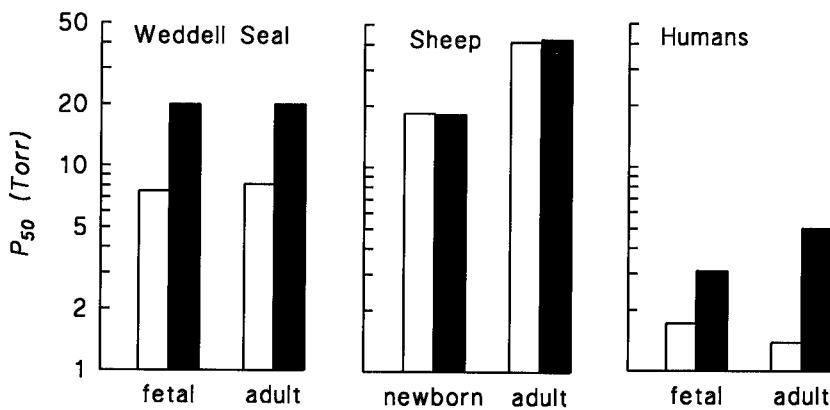


Figure 5 O_2 affinity (P_{50}) of fetal and adult Hbs from Weddell seal, sheep and humans, in the absence (open columns) and presence (solid columns) of DPG. [Weddell seal Hb, 37°C, pH 7.2, DPG/Hb molar ratio 7 (from ref. 34); sheep Hb, 37°C, pH 7.2, 40 torr CO_2 , DPG/Hb 2 (from ref 4); Human Hb, 15°C, pH 7.2; DPG/Hb 1 (from ref 40)].

(a) Hb switching

In sheep that express HbA, anemia induces synthesis of HbC, which has a higher O_2 affinity⁷. However, in goats HbC has the same affinity as HbA in the absence of CO_2 and a lower affinity in its presence⁴⁹.

Adults of the high altitude tolerant alpacas have high HbF levels (55%)³⁵. In the yak Hb that commonly express two adult and two fetal Hbs, fetal pigments with high O_2 affinities persist in the calves⁴⁵. Interestingly hypoxic exposure of pregnant ewes induces maintenance of HbF synthesis in the fetuses, whereas it decreases with time

in normoxic controls¹. Also, hypoxia increases fetal Hb synthesis in children with congenital heart disease².

Hb switching may also be manifested in birds, where some species, including all those known to fly at high altitude, express HbD whose fractional contribution may change drastically; in one individual Andean goose it fell from 40 to 4% within 3 years¹³. This isoHb shares common β chains with HbA whereby functional differences are due its α -chain sequences that show resemblance to those of avian embryonic Hbs. The high intrinsic Hb- O_2 affinity and low sensitivity to IHP (inositol hexaphosphate that closely resembles IPP) in a strain of high altitude chickens provide further evidence of molecular adaptation²².

(b) Gestational changes

Functional differentiation in fetal and embryonic Hbs commonly involves amino acid substitutions at the sites that are implicated in effector binding or in the transition between the T and R states of the Hb molecule.

In humans the high fetal blood O_2 affinity results from decreased DPG sensitivity in HbF caused by the $\beta 143\text{-His} \rightarrow \gamma 143\text{-Ser}$ replacement (that deletes two DPG binding sites per tetramer, Fig. 3) and substitution of $\beta 1\text{-Val}$ by partly acetylated Gly in the γ chains¹⁹. This contrasts with sheep (strategy II) where DPG insensitivity of the fetal Hb (as in adult ruminant Hbs—see below) results from deletion of the first β -chain residue and replacement of the second by non-basic methionine.

Human embryos (in the first ten weeks of pregnancy) have three embryonic Hbs known as Gower II, Gower I and Portland ($\alpha_2\epsilon_2$, $\zeta_2\epsilon_2$ and $\zeta_2\gamma_2$ where ζ and ϵ are embryonic α and β type chains, respectively. (Fig. 4^{6,42}). Hb Portland has a higher O_2 affinity than HbA, and a small Bohr effect, which tallies with the presence of acetylated serine at $\zeta 1$ (i.e. loss of a proton binding site compared to α chains). Moreover, having γ -chains as β -type chains (as in fetal HbF) predicts a reduced DPG effect in this Hb.

Pigs that lack fetal Hbs express Gower I and II (as in man) as well as Heide I and II ($\zeta_2\theta_2$ and $\alpha_2\theta_2$) and HbA during embryonic development (Fig. 4). Hbs Gower I and Heide I that have the highest O_2 affinities and lowest Bohr effects are the most abundant components in the earliest stages are successively replaced by Gower II and Heide II, and by HbA, which has the lowest affinity and the highest Bohr factor and becomes the dominant component when the embryos become about 4 cm crown-rump length⁴³. These findings indicate a progressive decrease in the O_2 affinity difference between maternal and embryonic blood as embryonic O_2 tensions increase in parallel with placental development⁴³. As with human Hb Portland (above) small Bohr effects in Gower I and Heide I are consistent with acetylation of the $\zeta 1$ residues. The higher O_2 affinity observed in Gower I and Heide I that have embryonic α chains (ζ) than in Gower II and Heide II with adult α chains appears to be due to the $\alpha 130\text{Ala} \rightarrow \zeta\text{Thr}$ replacement (as in adult vicuna Hb, below), which introduces a hydroxyl polar group that may increase O_2 affinity by interfering with Cl binding at adjacent $\alpha 131\text{-Ser}$ ^{18,42,43}.

Of the three embryonic mouse isoHbs investigated, $\chi_2\epsilon^Y_2$, $\alpha_2\epsilon^Y_2$ and $\alpha_2\epsilon^Y_2$ (where the χ subunits may actually be ζ subunits)^{6,15}, the first-mentioned lacks a Bohr effect and cooperativity and may function as a short-term O_2 store⁴⁸. In rabbits the embryonic Hbs with embryonic α chains similarly have higher O_2 affinities and lower pH sensitivities than those with adult type α chains^{15,42}.

A different situation applies to some marsupials, which are born at a very immature stage (when the blood still contains embryonic red cells) and continue develop-

ment without maternal placental connections in the mother's pouch. In Tammar Wallaby and brushtail possum the embryonic blood exhibits substantially *lower* O₂ affinities than adult blood, heterogeneous binding curves (with high cooperativity coefficients at high O₂ saturations) and contain multiple Hbs⁴² whose primary structures appear to be unknown.

2. Interspecific Adaptations

As with fetal and embryonic Hbs, the molecular adaptations in adult Hbs of high altitude species commonly entail replacements of the basic amino acid residues at the DPG binding site by nonpolar or hydrophobic ones, which lowers the stability of the Hb-DPG complex³².

(a) Mammals

Blood O₂ affinity of elephants, which may be encountered at altitudes of 4500m and were used in Hannibal's crossing of the Alps in the Second Punic War in 218 BC, is as high as in llamas¹². As in llama, vicuna and alpaca, elephant Hb exhibits a $\beta 2\text{-His} \rightarrow \text{Asn}$ substitution compared to human HbA^{18,19}, which deletes two of the seven DPG binding sites and diminishes the effector-induced reduction in O₂ affinity (Fig. 3). At pH 7.2 llama deoxyHb has a three-fold lower DPG binding constant than camel Hb³. Vicuna Hb that has even higher O₂ affinity shows a $\alpha 130\text{Ala} \rightarrow \text{Thr}$ exchange, which introduces a hydroxyl polar group that may interfere with Cl interaction at the adjacent $\alpha 131\text{-Ser}$ ¹⁸, i.e. the same adaptation seen in pig embryonic Hbs Gower I and Heide I (above).

Hbs from feloid and ruminant mammals have strongly reduced phosphate sensitivities⁷ indicating a reduced potential for adaptive O₂ affinity modulation. In bovine Hb the molecular basis is deletion of the $\beta 1$ residue and a $\beta 2\text{-His} \rightarrow \text{Met}$ replacement, which obliterates 4 of the 7 DPG sites (Fig. 3). The hydrophobic $\beta 2\text{-Met}$ residue is forced to the interior of the molecule and stabilizes the low-affinity T structure²⁸. In cat HbA and HbB exhibit a $\beta 2\text{-His} \rightarrow \text{Phe}$ substitution, whereas the $\beta 1$ residue in HbB moreover is an acetylated serine that cannot bind DPG^{7,19}.

b. Birds

Although birds generally may be considered preadapted to hypoxia through unique adaptations at the systems level, distinguishing adaptations at the molecular level endow some species with critical advantages in tolerating extreme hypoxia²³.

As with elephants, the high blood O₂ affinity in flightless ostriches and rheas accords with lower weight-specific O₂ demand and O₂ unloading in the tissues of large animals, and with replacement of $\beta 2\text{-His}$ (see ref. 26 and Fig. 3). In ostriches this tallies with the use of ITP (inositol tetraphosphate) instead of IPP as affinity modulator¹⁶. The same $\beta 2\text{-His} \rightarrow \text{Gln}$ substitution is manifested in swift, starling and blackbird²⁵, suggesting high, intrinsic O₂ affinities in Hbs from these species.

In contrast to other birds that have one or two isoHbs, Ruppell's griffon that can fly at 11.3 km has four Hbs, viz., HbA, HbA', HbD and HbD' that exhibit cascaded O₂ affinities (lowest in HbA and highest in D/D')⁴⁴. This differentiation is consistent with substitutions at $\alpha 34$ that stabilize the T-structure in HbA or at $\alpha 38$ that stabilize the R-structure in Hbs D/D'¹⁴ and suggests functioning of the Hbs over a wide range of O₂ tensions.

The bar-headed goose; a case study in molecular adaptation

One of the most intensively studied high altitude tolerant birds is the bar-headed goose. Specimens acclimated to a simulated altitude of 5.6 km and subjected to 11.58

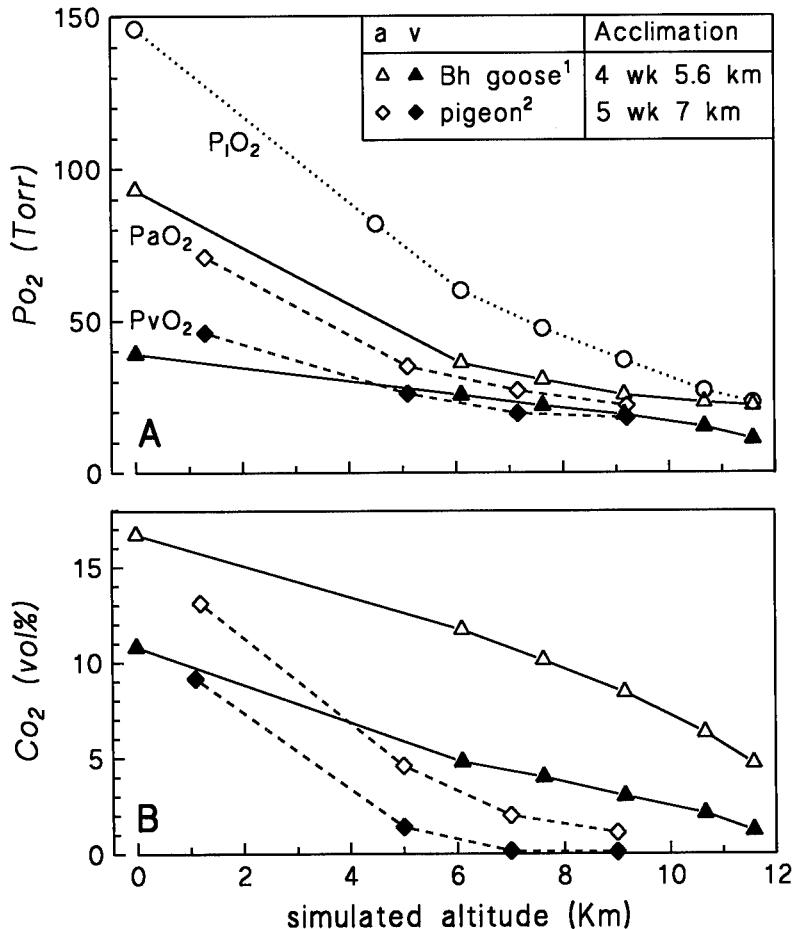


Figure 6 A, Inhale O₂ tensions (P_iO₂, circles) at different simulated altitudes and arterial (open symbols) and venous (closed symbols) O₂ tensions in bar-headed goose (triangles) and pigeons (diamonds), showing that arterial O₂ tension in the bar-headed goose at 11.58 km is only 1 torr below that in inhale air, and B, corresponding arterial and venous O₂ content values, showing higher values in bar-headed goose. Data from refs. 5 and 17.

km maintain an arterial O₂ tension that is only 1 torr lower than in inhaled air (Fig. 6) and an arterial O₂ saturation of 28%, compared to corresponding values of 4 torr and 2% saturation in Pekin ducks⁵. These findings tally with a high blood O₂ affinity compared to that in the greylag goose that colonizes lower planes (P₅₀ at 37°C and pH 7.4 = 29.7 and 39.5 torr, respectively)²⁹. This difference cannot be explained by differences in red cell IPP levels or the reactivities of the Hbs with IPP, but correlates with a small difference in the intrinsic affinities of the Hbs ($\Delta P_{50} = 3$ torr at pH 7.2 and 37°C) that becomes strongly magnified in the presence of red cell effectors^{29,37}.

The primary structures of the Hbs from the bar-headed and greylag geese show only four exchanges^{19,26} of which one, viz., α 199-Pro \rightarrow Ala is unique amongst birds and mammals suggesting its involvement in high altitude adaptation. In human HbA, α 199-Pro forms a Van der Waal contact with β 55-Ser that stabilizes its T-structure (reducing O₂ affinity). The theory that loss of this single $\alpha_1\beta_1$ contact may be basic to

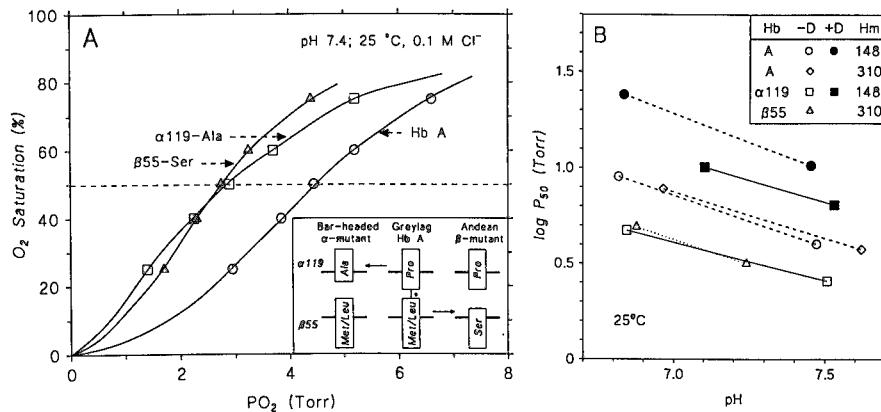


Figure 7 A, O₂ equilibrium curves of native human HbA (circles) and of mutant Hbs α119Ala and β55Ser created with site-directed mutagenesis to mimic mutations manifested at these sites in the Hbs from bar-headed and Andean geese (see inset). B, pH dependence of P₅₀ values (Bohr effects) of Hbs A (circles), α119Ala (squares) and β55Ser (triangles) in the absence (open symbols) and presence (solid symbols) of DPG (-D and +D, respectively) at 25°C and the indicated heme concentrations. Modified after ref. 46.

the exceptional altitude tolerance in geese, is supported by the fact that the Andean goose that also has extremely high blood O₂ affinity shows a β55-Leu→Ser substitution, and that both Pro and Ser are short residues that would leave a gap between α119 and β55 (Fig. 7). This hypothesis was tested by engineering two human Hb mutants that mimic the evolutionary changes that occurred in the bar-headed and Andean geese (α119-Pro→Ala and β55-Met→Ser) by site directed mutagenesis and comparing their O₂ affinities with those of human HbA^{17,46}.

The two mutants showed similar increases in O₂ affinities compared to HbA, which is compatible with loss of the same (α119-β55) intramolecular T-state contact (Fig. 7). Although the P₅₀ difference compared to HbA was small it was drastically increased in the presence of DPG (from approximately 2 to 17 Torr at pH 7.4 and 37°C)⁴⁶, as with Hbs from the bar-headed and greylag geese (see above).

Measurement of precise O₂ equilibria of the Hbs at extreme (low and high) saturations (Fig. 8) and mathematical analysis in terms of the Monod-Wyman-Changeux two-state allosteric model permits evaluation of the affinity constants for the Hb in the tense and relaxed states (K_T and K_R, respectively) and for the four successive oxygenation steps (k₁ - k₄)⁴⁶. The latter ('Adair') constants reflect higher affinities for binding the first three O₂ molecules in the α chain mutant than in HbA both in the absence or presence of cofactor, and indicate that, in both molecules, DPG delays the transition from the T to the R state until binding of the third O₂ molecule.

The α119-Ala mutant showed a significantly higher K_T value than HbA (Fig. 8), indicating lower bond energies constraining it in the T state. Calculated from the respective K_T values (which can be determined more accurately than the K_R values) the difference in this bond energy between the mutant and native Hb molecules approximates 2.4 and 3.3 kJ in the absence and presence of DPG, respectively⁴⁶. These values are within the range of stabilization energies for Van der Waals interactions (0.4 - 4.0 kJ/mole, compared to 12-30 kJ/mol for hydrogen bonds¹⁰). This supports the hypothesis that loss of a single intramolecular contact underlies the increase in

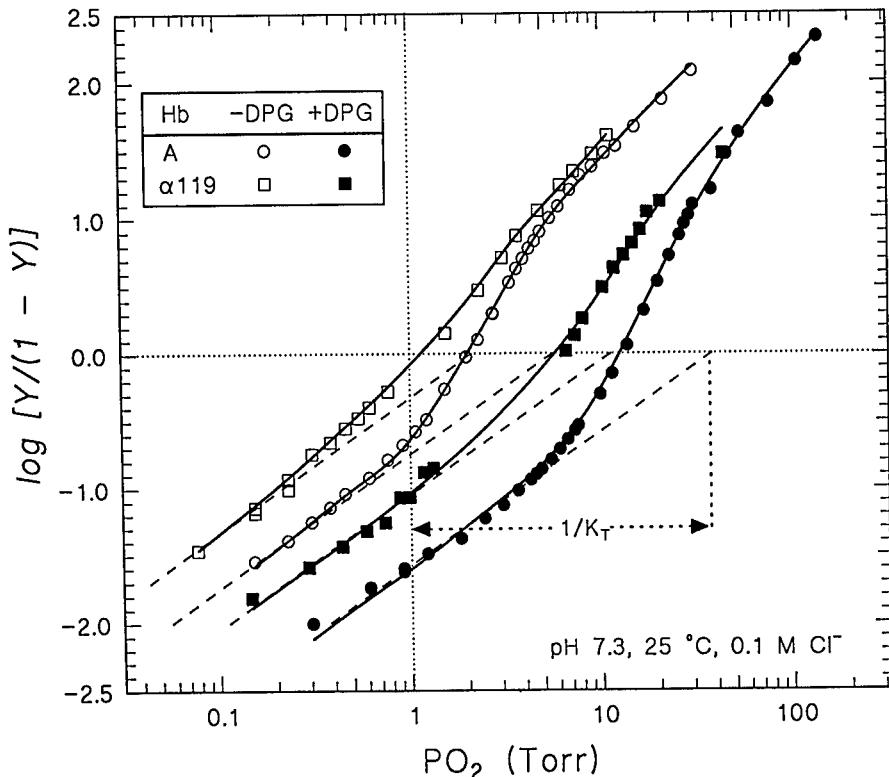


Figure 8 Extended Hill plots of human HbA (circles) and mutant Hb α 119Ala (squares) in the absence (open symbols) and presence (solid symbols) of DPG at 25°C and pH 7.3, illustrating shifts in K_T (the O_2 association constant of the deoxygenated, tense state), which suggest that loss of a single intramolecular contact (α 119- β 55) causes the high intrinsic O_2 affinity of bar-headed goose Hb.

intrinsic Hb- O_2 affinity, which endows the bar-headed and Andean geese with a selective advantage to exploit extreme high altitude niches. These considerations predict a similar, high intrinsic O_2 affinity in other Hbs lacking a α 119- β 55 contact, such as in Hb of the white stork (*Ciconia ciconia*) which exhibits β 55-Thr¹¹ that resembles Ser found in the Andean goose. It is remarkable that single point mutations occurred at the same contact but on different globin chains in these two geographically widely separated species^{17,46}.

It should, however, be stressed that adaptations in Hb structure/function are only part of a range of molecular, cellular and organismic adaptations that enables respiration at extreme altitudes. Thus hyperventilation-induced alkalosis^{5,47} may raise blood affinity via the Bohr effect and decreased Hb-IPP complexing. Also, as shown for pigeons³⁰ cooling of blood in the heat-exchanging ophthalmic retia and exchange of CO_2 and O_2 between air and blood at moist cephalic surfaces may substantially increase the O_2 affinity and O_2 content of afferent cranial blood.

D: Conclusion

High altitude tolerant birds and mammals exhibit two main strategies for securing O_2 delivery in tissues, (a) intraspecific changes in red cell concentrations of phos-

phate cofactors (IPP and DPG), as illustrated in mammals subjected to moderate hypoxia, and (b) synthesis of isoHbs with different structural and functional properties, as observed with intraspecific Hb switching and gestational development, and with interspecific comparisons.

Molecular adaptation is not a general characteristic but reflects specific mutations that distinguish species with exceptional hypoxic tolerance and complements general adaptations to hypoxia at the cellular and systems levels (e.g. capacity to regulate red cell phosphate levels in mammals and the unique functional anatomy of the lung in birds).

The involvement of only a few amino acid residues in the intramolecular interactions that determine Hb-O₂ affinity limits the options available for molecular adaptations and is the basis for the homology in strategies encountered in adult, fetal and embryonic Hbs from phylogenetically diverse species. Thus, a reduction in the modulator action of phosphates is achieved by replacing the same basic β 2-His residue by uncharged Asn (in llama, vicuna, alpaca and elephant), Gln (in ostrich and swift), Phe (in cat) or Met (in ungulates like cow, goat and sheep).

The use of site-directed mutagenesis to create Hbs with key amino acid exchanges that mimic those evident in the Hbs of high altitude tolerant geese show that pin-point mutations that change single intramolecular contacts may impart critical selective advantage for high altitude respiration. Such investigations provide for exciting perspectives for designing Hbs adapted to hypoxia or specific pathological conditions, given the development of technologies that permit *in vivo* expression of engineered Hb mutants.

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CHAPTER 5

EARLY DEVELOPMENT OF BLOOD OXYGEN TRANSPORT

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Abstract

During the early embryonic phases of all vertebrates, gas exchange and distribution within the developing tissues by simple diffusion is gradually supplanted by convective transport of oxygen using a respiratory pigment distributed by the action of the heart. It has been widely assumed that the beat of the heart and subsequent convective flow of blood begins just as diffusion becomes inadequate because of lengthening diffusion distances and increasing metabolic rates. This hypothesis, which we call *synchronotropy*, has not been extensively tested. Two distinct lines of evidence, however, support the hypothesis of *prosynchronotropy*, which proposes that the heart beat and convective flow of blood begins before the absolute requirement for convective blood oxygen transport. The first evidence comes from experiments in which embryonic fish larvae²¹ and amphibian larvae (Territo, unpubl., Mellish *et al.*, unpub.) are exposed to 2-5% CO. Convection of blood by the beating heart continues, but Hb-O₂ transport is eliminated. Oxygen consumption is not significantly affected and the larvae even continue to grow and develop, suggesting that convective O₂ delivery by Hb is not essential. The second line of evidence comes from larval axolotls, either of a mutant strain in which the heart never begins to beat,²⁵ or in wild type larvae in which the heart primordia had been removed earlier by surgery²⁹. Larvae with no functional cardiac pump for generating convective blood flow show only slightly diminished oxygen consumption compared to wild type larvae. Collectively, these data suggest that the vertebrate heart starts to beat and pump blood well before convective blood oxygen transport is actually required.

Introduction

A major tenet in cardio-respiratory physiology is that the convective movement of blood provided by the heart is required to distribute oxygenated hemoglobin (Hb) about the body. This statement is fundamentally true for juvenile and adult animals, but requires some refinement when placed in an ontogenetic context. During the earliest embryonic phases of all vertebrates, gas exchange and distribution within the developing tissues must occur by simple diffusion, for there is no mechanism for convective transport of blood and, indeed, there is no blood. Then, as the heart begins to beat, erythrocytes with Hb begin to form. Blood begins to flow to the peripheral embryonic tissues, and convective transport of oxygen using a respiratory pigment gradually supplants diffusion¹¹.

Unfortunately, we know surprisingly little about the timing of the onset of Hb-oxygen transport in these earliest stages of development. The literature on cardio-respiratory physiology and blood oxygen transport in the developing mammalian *fetus* is enormous³⁹, but we know little about blood gas transport in the mammalian *embryo*²². For a number of reasons, both technical and physiological, mammalian embryos are not easily studied. To give just one example, the mammalian embryo prior to placental development is highly protected within a uterine environment that, with respect to PO_2 and PCO_2 , is poorly understood and characterized and correspondingly difficult to modify experimentally. Because of these and other limitations, cardio-respiratory physiologists interested in the developmental onset of blood oxygen transport have turned to other vertebrate embryos that are more conducive to study. The two most widely employed are the chicken embryo and, more recently, the frog embryo (for a discussion of the merits of these models and data derived from them see numerous articles^{6,11,12,26,36} and the Israel J. of Zoology Vol. 40, 1994.) Briefly, however, both models offer similar advantages over studying mammalian embryos because these embryos are, by nature, self-enclosed entities free of direct maternal influence. Although both bird and amphibian embryos are enclosed within specialized eggs of some description, the experimenter can relatively easily modify the immediate embryonic environment, and can access embryonic structures more easily, with less embryonic disruption, than in mammalian embryos. Ease of access is probably maximized in many amphibian and fish embryos, which hatch from their egg as larvae at about the period of time when the heart begins to beat. Since many of these embryos are also nearly transparent, video microscopy and other techniques can be employed to make both qualitative and quantitative measurements of cardio-respiratory processes⁹.

Although the study of blood oxygen transport and tissue oxygenation in developing vertebrate embryos is still in its infancy, there are sufficient data available to describe the basic process, to speculate on some as yet unexplored processes and, in some instances, modify and refine our view of the role of embryonic hemoglobins. We begin by considering the role of diffusion in the earliest embryonic stages.

Diffusion: The Earliest Mechanism for Embryonic Oxygen Transport

Diffusion as the sole mechanism for providing oxygen transport between the environment in which an animal lives to the mitochondria of its cells is surprisingly effective provided three conditions are met:

- total distances for gas diffusion are short
- environmental PO_2 's create large gradients for inward oxygen flow
- tissue metabolic rates are low

Some, if not all, of these conditions are relevant to early embryonic stages of vertebrates, and each deserves discussion in turn.

Short Diffusion Distances

Living proof abounds of the efficacy of oxygen diffusion without convective transport due to small body size. There are numerous invertebrates that as adults have no cardiovascular system and no blood pigment. Such animals are usually limited to about 1 mm in diameter, resulting in a maximum possible oxygen diffusion distance of about 0.5 mm. However, specialized body shapes can permit larger body sizes. The flatworm *Planaria* and similar *Platyhelminthes* can reach several cm in length and a

cm or more in width. They survive without a cardiovascular system and the attendant convective oxygen transport largely because their flat body shape ensures that all body cells are within 0.5 mm or so of the water in which they live. Even in vertebrates, there are qualified examples of animals that survive with no convective transport of oxygen in blood. As will be discussed below, a mutant form of the axolotl *Ambystoma mexicanum* produces embryos that survive to several mm in length without a beating heart before eventually succumbing.

Since all vertebrate embryos begin as just a pair of fused cells, early development is characterized by short diffusion distances, even when accounting for the yolk mass of the egg. The cardiovascular system is the first system to function in the vertebrate embryo, but convective flow of blood with erythrocytes still lags behind the initial explosive period of embryonic growth. Consider the human fetus. The theoretical maximum diffusion distance of 0.5 mm between deepest tissues and the surrounding environment is not exceeded until about 20-24 days of development. This coincides with the beginning of an organized convective flow of blood generated by the beating heart.

Large PO₂ Gradients

Diffusion as the sole mechanism for delivering oxygen to embryonic tissues is effective only if the PO₂ gradient from environment to metabolizing tissue is large. Assuming that the PO₂ within the mitochondria is less than 0.5 mmHg⁴¹, then the size of the oxygen gradient will be dictated by ambient PO₂, and changes in the rate of oxygen diffusion into the embryo will similarly depend upon changes in ambient PO₂. This being the case, what do we know about the PO₂s in the embryonic environment? Answering this question depends upon the taxon being considered.

In non-marsupial mammals, the embryo implants into the uterine wall, which subsequently grows around it. Data on the oxygen characteristics of the environment immediately surrounding the embryo are lacking—we know of no measurements of PO₂s in the immediate vicinity of the embryo in the first few weeks of pregnancy. In the absence of data, one might speculate that such PO₂s approximate that of mixed venous blood, in the order of 40 mmHg. This makes for a PO₂ gradient of about 40 mmHg between “environment” and mitochondria. Bird embryos and reptile embryos are separated from ambient air by calcified egg shells representing potential boundaries to diffusion^{13,32}. The diffusion properties of bird and reptile egg shells is complex, because of the geometry of the egg shell pores, the effect of the water that occupies these pores, whether they are calcareous or leathery eggs, and the properties of the underlying membranes. However, at least in chicken eggs, the PO₂ in the air cell inside the egg is between 100 and 110 mmHg³². The embryo itself begins to form close to the inner membranes of the egg (that is on the exterior of the yolk mass), and may experience similarly high “environmental” PO₂s in the first days of development.

Amphibian and fish embryos form in eggs that as a general rule contain large amounts of perivitelline fluid relative to bird and reptile eggs. Moreover, depending on species, the eggs themselves may be imbedded in large amounts of jelly-like material^{33,37}. This jelly can present an additional large diffusional barrier, roughly equivalent to the boundary layer of unstirred water. Though beyond the scope of this article, it is worth mentioning that the situation in some amphibian egg masses is made even more complex by the presence of photosynthesizing algae that contribute oxygen to

the egg mass during the day, and consume it at night³³. Ignoring problems for gas diffusion presented by the additional jelly, the eggs of many species of amphibians and fishes are at least 1 mm in diameter and up to several mm in diameter. This would appear to obviate adequate inward oxygen diffusion to supply the metabolism of the growing embryo. However, at least in some amphibian eggs the body wall of the early embryo is ciliated. These cilia beat in a coordinated fashion, generating a convective flow of perivitelline fluid within the egg and reducing the boundary layer adjacent to the skin⁸. Thus, the appropriate gradient (and diffusion distance) to consider in these situations is from the perivitelline fluid adjacent to the body wall to the deepest tissues. Unfortunately, actual measurements of PO_2 in or around amphibian or fish eggs are few^{8,33,37}. However, modeling has provided a useful, if controversial, approach to understanding diffusional gas transport in amphibians^{35,37}.

Low Metabolic Rates

The extent to which the embryo acts as an oxygen sink is a crucial consideration in the effectiveness of gas diffusion as the sole means of providing oxygen to the embryo. There are numerous studies of the metabolic rate of non-mammalian vertebrate embryos (for reviews see 10, 34, numerous papers in Israel J. Zool. vol 40, 1994). Unfortunately, most data suffer from the same flaw—the measurements were made on organisms whose body mass included large and variable amounts of non-metabolizing tissues in the form of yolk. Oxygen consumption of embryos is usually expressed as oxygen consumed per embryo or as oxygen consumed per gram of embryo. This latter measurement may include large amounts of perivitelline fluid and membranes (fish, amphibians), or calcareous egg shells and massive amounts of yolk (reptiles and birds). Only rarely have attempts been made to express embryonic oxygen consumption based on some index related to metabolizing tissue. These indices have included nitrogen, DNA, body mass after ethanol extraction of yolk, and lipids. Currently, we are developing a technique in our lab which would allow for separation of the polar and non-polar fraction of the yolk mass. The novelty about this refined method of determination is that it utilizes HPLC to quantify the fraction of each type of lipid present, and therefore to determine which portion of the egg is “animal” and which part is stored fuel.

Given the difficulty in expressing oxygen consumption of embryos in a meaningful way, it is not surprising that no clear indication of embryonic metabolic rate exists. Assumptions that embryos have the same metabolic rate as adults may be in error because of the allometry of metabolism and because of non-intuitive changes in metabolic rate during development^{17,18,31}. However, even if embryonic metabolism is over- or under-estimated grossly, the fact that most vertebrate embryos are in the order of a few mgs would indicate that large scale oxygen depletion of the environment surrounding the embryo is unlikely. This is not to say that steep PO_2 gradients may not exist³⁵, but rather that even a high metabolic rate by an embryo is unlikely to outstrip diffusional delivery of oxygen while diffusion distances remain short.

Onset of Convective Transport of Oxygen in Blood

As outlined above, oxygen transport by diffusion can satisfy the metabolism of embryos when diffusion distances are small, most vertebrate embryos quickly grow beyond a few mm in size. Regardless of changes in ambient PO_2 and metabolic rate, increasing diffusion distance obviates diffusion as a sole means of achieving tissue

oxygenation. This necessitates the convective flow of an oxygen-carrying medium—blood containing Hb packaged in erythrocytes in the case of vertebrates.

Heretofore it has been assumed that the onset of convection of blood to transport respiratory gases (and nutrients) occurs just as diffusional delivery of oxygen begins to falter. However, a growing body of evidence suggests that blood convection may be only a prelude—dress rehearsal, if you will—to the actual need for convective oxygen transport. We can express these two different interpretations formally:

Hypothesis #1. The heart, heart beat, and blood flow develop on a “just in time” basis to supply by convection oxygen to growing embryonic tissues as simple diffusion of O_2 becomes progressively insufficient.

or

Hypothesis #2. Convective flow of blood generated by beating of the developing heart precedes the absolute need for convective flow although diffusion is adequate to meet animal’s needs during the period of early heart development. That is, early heart development is “dress rehearsal” for a future time when convective blood becomes an absolute requirement.

Figure 1 illustrates these two hypotheses. Let us now consider the evidence for each hypothesis.

The “Synchronotropic Hypothesis”

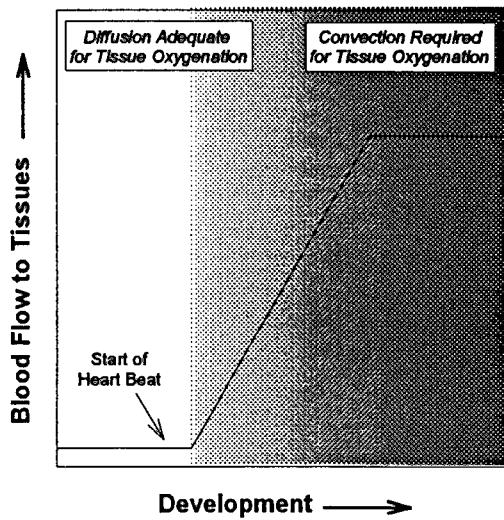
The “synchronotropic hypothesis”, which we might call the “just in time” hypothesis, has not been explicitly tested experimentally to our knowledge. This hypothesis derives less from any experimental data than from a long-standing, central dogma in biology, namely, that extant structures carry out functions useful to the animal. We generally assume that when structures are present and in a non-diseased state, they do, in fact, perform their ascribed function (with the exception of vestigial organs, of course). Because the heart begins to beat and generate convective blood flow early in development, and because intuitively we know that the growing embryo will at some time require a system of internal convection for oxygen and nutrient distribution, it is widely assumed that the heart beat appears at the time when such convection is required (Fig. 1A).

This reasoning is somewhat flawed, given what we know about physiological and anatomical developmental processes for other organ systems. The lungs, kidney, liver and other organs of the mammalian fetus develop and show physiological capabilities well before normal events in development require them to operate. We know, for example, that fetal lungs show some physiological capabilities in the form of breathing movements and the ability to exchange gases (as proven by premature birth) weeks or months before the act of birth at normal term makes sudden demands for gas exchange. Why then, should we not similarly assume that the beat of the heart and the convective flow of blood represents preparation for, rather than the actual need of, blood oxygen transport? Part of this preparation for the eventual use of the circulation or O_2 transport could include the hemodynamic modeling and shaping of the heart and vessels by the heart beat.

The “Prosynchronotropic Hypothesis”

The “prosynchronotropic hypothesis” or “dress rehearsal” hypothesis suggests that, like fetal lungs that show breathing movements but are not actually exchanging gases, the early embryonic heart beat and blood flow that it generates are preludes to a

A

Hypothesis 1: Synchronotropy

B

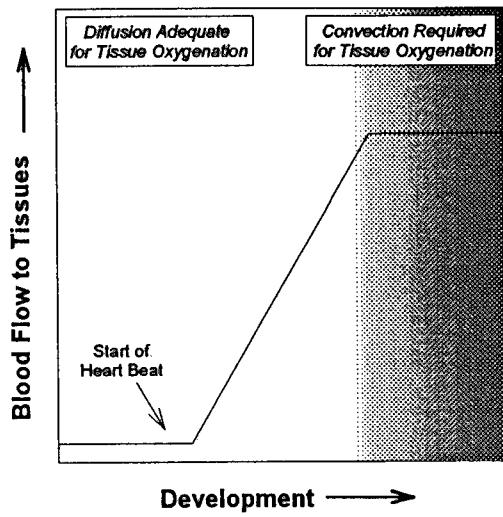
Hypothesis 2: Prosynchronotropy

Figure 1 Contrasting hypotheses describing the timing of the onset of heart beat relative to the need for convective transport of oxygen by blood. A) The “synchronotropic hypothesis” holds that the onset of heart beat (and blood convection) is synchronized with the point in development when diffusion becomes insufficient for gas exchange. B) The “prosynchronotropic hypothesis” holds that the need for convective blood flow to transport oxygen to the tissues does not occur until well after heart beat and blood flow have begun.

period when convective flow of blood will be required for gas exchange early in embryonic development. This hypothesis is currently being tested in a number of laboratories using three experimental paradigms—non-lethal effects of carbon monoxide in embryos, surgical removal of the heart primordia and the cardiac lethal mutant line of axolotls (*Ambystoma mexicanum*).

Carbon Monoxide in Embryos—Disruptive, But Not Deadly

A wide variety of vertebrates possesses multiple forms of embryonic or fetal respiratory pigments^{3,27,40}. Convective transport of these pigmented cells facilitates the complete transport of oxygen, and therefore ultimately permits cellular respiration, at relatively low ambient PO_2 s. A standard tool in investigating the role of hemoglobin specifically, and blood generally, in animals of all developmental stages is “functional” ablation of Hb with carbon monoxide (CO). The importance of utilizing CO as a means of lowering oxygen content is that CO:

- is specific to Hb;
- does not interfere with carbonic anhydrase activity, which is important in CO_2 transport and buffering;
- does not reduce whole blood viscosity, as in the case of anemia, and;
- allows the investigator to determine the role of Hb in bulk oxygen transport.

Carbon monoxide as a probe of Hb oxygen transport was used extensively in mammals, especially in the '70s^{2,4,5,14}. However, use of CO to investigate O_2 -Hb transport in embryonic or larval vertebrates has been limited to a few studies on fishes²⁰ and amphibians (Mellish *et al.* unpubl.; P. Territo, unpubl.). Nonetheless, the data are provocative, and bear discussion because of the relevance they have to the “dress rehearsal” hypothesis.

Holeton²¹ was one of the first to expose vertebrates in very early stages of development to CO. He briefly exposed one month old larvae of the rainbow trout *Salmo gairdneri* to 5% CO and monitored branchial pressure, heart rate, and oxygen consumption. Holeton²¹ determined that larval rainbow trout could survive brief exposure to 5% CO with few ill effects, although respiratory and heart rates were elevated over this exposure. Moreover, this CO level was found to reduce maximum blood oxygen content to 0.8 vol %, compared to 9 vol% in air-saturated blood in adults of the same species²⁰. This level of CO is sufficient to limit the oxygen carrying capacity of Hb, but does not affect other hemoproteins such as cytochromes¹⁵. Additionally, a P_{CO} 5-10 x greater than PO_2 would be required to reduce oxidative metabolism by 50-80%, due to a direct effect of CO on cell metabolism. Clearly, these lines of evidence demonstrate that blood-oxygen transport is unnecessary for gas exchange in rainbow trout, a fish with a relatively high metabolic rate.

The metabolic effects of CO exposure are also being investigated in amphibian larvae. Mellish *et al.* (unpublished) have investigated the effects of CO exposure in amphibian larvae (Fig. 2). They exposed stage 39 larval axolotls (*Ambystoma mexicanum*) to 5% CO. Larval axolotls at this stage have developed external gills, and their heart has been beating and propelling blood containing erythrocytes for several days. Oxygen consumption (\dot{MO}_2) was decreased by only about 25% in CO-exposed stage 39 larvae compared with normal larvae. Oxygen conductance (\dot{GO}_2) in these animals was similarly reduced. Collectively, these data indicate that axolotl larvae can consume oxygen with relatively little assistance from Hb as a blood oxygen carrier.

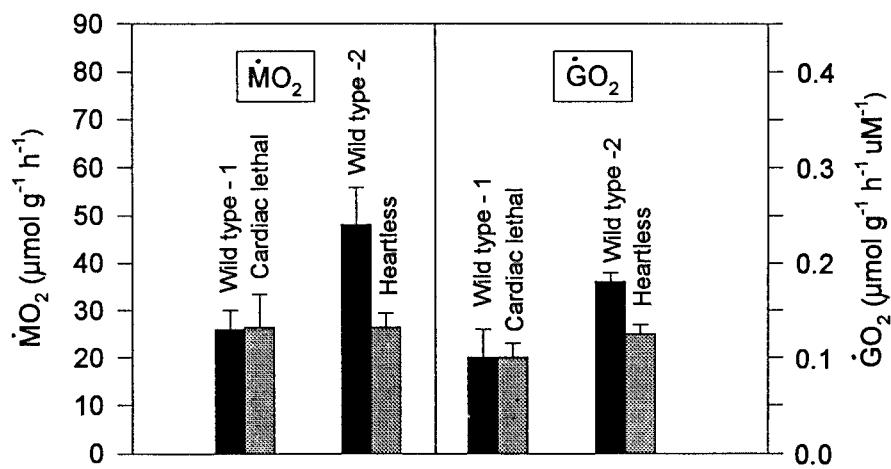


Figure 2 Oxygen consumption ($\dot{M}O_2$) and oxygen conductance ($\dot{G}O_2$) in stage 39 larvae of the axolotl *Ambystoma mexicanum* in normoxia. Two experiments are indicated. In the first group, a population of wild type larvae were compared with a population of cardiac lethal mutants. In the second group, a different population of wild type larvae were compared with wild type larvae in which the heart primordia had been removed earlier at Stage 18-20. After Mellish, Smith and Pinder, unpubl.

Recent studies in our own lab have concentrated on the long term developmental, metabolic and respiratory effects of 2% CO in the South African clawed frog *Xenopus laevis*. This level of CO reduces maximum blood oxygen content to 0.2 vol % from untreated levels of about 9-10 vol %, a CO effect consistent with Holeton²¹ findings for larval trout. In our study we measured $\dot{M}O_2$, whole body lactate, mass, and developmental stage during growth and development in 2% CO. Although discussion of many of our findings is beyond the scope of this paper, the fundamentally important finding is that CO-exposed *Xenopus laevis* continued to consume oxygen at levels not significantly different from air-exposed larvae, at any stage of larval development (Fig. 3). Moreover, they continued to grow even when completely deprived of Hb-O₂ transport by CO exposure. The larvae also displayed normal feeding and respiratory behavior. In fact, the major impediment to development appeared to be a slowing in the rate of growth rather than the ability to grow.

Collectively these data from lower vertebrates indicate that Hb-dependent oxygen transport by the blood is unnecessary for survival and growth in early embryos and larvae. How do CO-exposed larvae continue to consume oxygen, grow and develop? One answer is that a greatly elevated cardiac output may transport sufficient oxygen dissolved in solution in plasma, when it cannot be bound to Hb. There are numerous species of vertebrates that have little or no Hb as adults. The Antarctic "icefishes" *Chaenocephalus aceratus*, *Pseudochaenichthys georgianus*, and *Champsocephalus gunnari* lack Hb, and rely solely on convective transport of dissolved oxygen^{19,28}. These fish are greatly aided by the fact that they live at or near 0°C, which greatly increases the solubility of oxygen in their plasma compared to warmer temperatures. The combination of high oxygen plasma content, large blood volume and a cardiac output that is about 6-15 times greater than other species of fish with Hb at this temperature would appear to allow adequate rates of oxygen consumption.

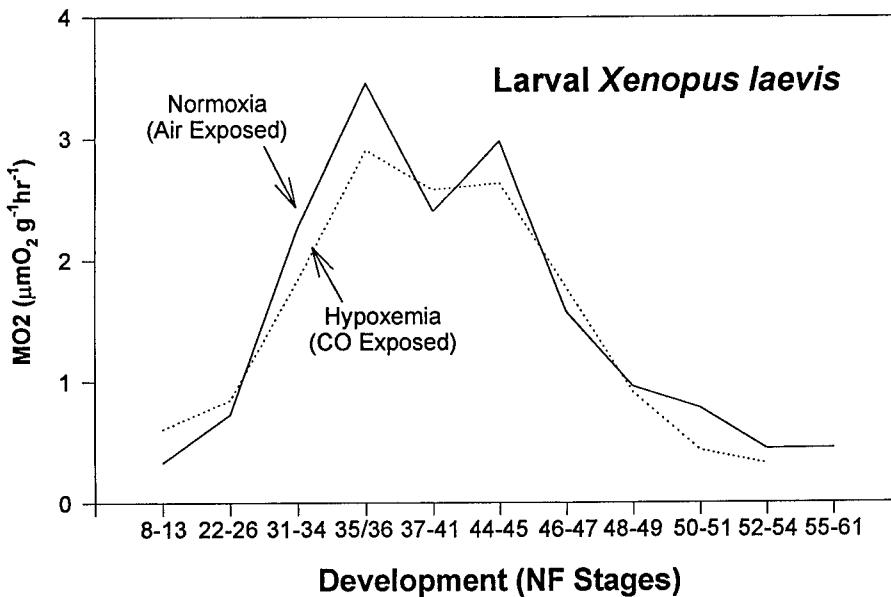


Figure 3 Oxygen consumption ($\dot{M}O_2$) at 23–25°C in larvae of the clawed frog *Xenopus laevis* as a function of development and of chronic exposure to 2% CO from egg fertilization. Note that the $\dot{M}O_2$ of the larvae chronically exposed to CO was not different from that of the larvae raised in normoxia. Development is expressed in Nieukoop-Faber stages. See text for more details (P. Territo, unpubl.)

CO-exposed salamander and frog larvae consume oxygen at rates at or slightly below animals with unimpaired Hb (Figs. 2, 3). Enhanced plasma O_2 carriage such as enjoyed by icefish cannot account for blood O_2 transport alone in larval amphibians. This is because there is nothing unusual about O_2 carriage in plasma. Experiments on *Ambystoma mexicanum* and *Xenopus laevis* were carried out at 20–25°C, and the O_2 plasma solubility in at least *Xenopus laevis* is comparable to mammals and other vertebrates (7, P. Territo, unpublished). Consequently, we predict that some combination of the following must occur to account for the maintained O_2 consumption:

- cardiac output is greatly elevated to provide O_2 by convection in plasma
- diffusion rather than blood convection provides most, if not all, O_2 required by metabolizing tissues.

These two opposing explanations have not been experimentally explored as yet. However, extremely compelling evidence supporting the notion that diffusion is adequate comes from experiments on cardiac lethal mutants of *Ambystoma mexicanum*.

“Heartless” Larvae—Survival by Diffusion Alone

There exists in the axolotl *Ambystoma mexicanum* a “cardiac lethal mutant”. The heart develops more or less normally from a gross anatomical perspective, but it never begins to beat (see reviews 16, 25, and 38). If the normal onset of heart beat occurs just as the need for convective blood transport develops—that is, if synchronotropy prevails—then cardiac lethal mutant embryos should die at the time when the heart of normal embryos begins to beat. In fact, cardiac lethal mutant embryos hatch into larvae and swim about for several days before succumbing—all the time with no

heart beat! In a recent series of experiments, Mellish, Pinder and Smith²⁹ and Mellish, Smith and Pinder (unpublished) have shown quite unequivocally that the heart is not required for survival in stage 39 axolotls. Their experiments were carried out on three larval populations—wild type axolotls (controls), wild type heartless (heart primordia surgically removed at stages 18-20), and cardiac lethal mutants with heart intact, but which by nature had no heart beat. They then measured $\dot{M}O_2$ and inward O_2 conductance in two experiments, using two separately raised clutches of larvae. As shown in Figure 2, there was little or no impairment of either $\dot{M}O_2$ or PO_2 conductance in animals with no heart beat or, in the more extreme case of the surgically treated population, no heart at all!

It is possible that some minor convective flow of blood could be generated in a cardiac lethal mutant, or even in a heartless wild type larvae, if there was an intact central circulation with venous valves. Small body movements could drive blood slowly through such a cardiovascular system even in the absence of a heart beat.

Conclusions, Implications and Future Directions

At some time in the early development of every vertebrate the ability of oxygen to reach the tissues by simple diffusion is outstripped by the oxygen demand of the tissues. At this time the convective transport of oxygen by blood must begin, and take on a progressively increasing role in overall tissue oxygenation as body mass and diffusion distances increase. Generally it has been assumed that the onset of heart beat, and the convective flow of blood that the beating heart provides, occurs just at the time when convective flow is needed, a hypothesis that we call synchronotropy. However, the alternative hypothesis of prosynchronotropy—that the heart starts to beat and pump blood before convective blood oxygen transport is actually required—is supported by some compelling experimental data on embryos and larvae of amphibian vertebrates. Elimination of $Hb-O_2$ transport by carbon monoxide exposure, cardiac lethal mutants lacking heart beat, and even surgical removal of the heart primordia is insufficient to prevent oxygen consumption, growth and development to a time in development well beyond that at which control animals develop heart beat and blood flow. Clearly, tissue oxygenation is not dependent on convective blood flow in early development. A natural question, then, is why *does* the heart begin to beat “early” with respect to the need for convective oxygen transport? Mellish *et al*²⁹ speculate that the heart may begin to beat “... for the removal of nitrogenous wastes, and/or to maintain ionic and water balance”. Generation of central arterial pressure might also be important in angiogenesis¹.

These experiments and their interpretation suggest several areas for future research. One of the most important to pursue will be to establish how universal these findings are among vertebrates. An important first step would be to investigate the onset of the need for convective transport of oxygen in the early chick embryo, which has been used widely as a model for human and mammalian embryonic development. However, we do know that, at least for many aspects of hemodynamic development, amphibian and avian embryos are qualitatively and in many respects quantitatively the same (see 9, 23, and 24). Another area to investigate will be whether oxygen transport by plasma plays a significant role in early embryos, when blood Hb concentration and red blood cell counts are very low. Related to this is the question of whether increases in cardiac output can compensate for the lack of functional Hb. These and other questions exploring the role of blood in tissue oxygenation in the early embryo

will probably be more easily explored by continuing to develop and work with cardiovascular and erythropoietic mutants of zebrafish, amphibians and chickens or other fowl.

Finally, understanding the role of the early circulation in vertebrate embryos is not just an academic exercise. Many surgical procedures are now carried out on the human fetus—procedures thought impossible just a few short years ago. The future may hold the promise of cardiovascular surgery during the first trimester of human pregnancy. Knowing the precise role of the embryonic and early fetal circulation will be crucial in making decisions about interrupting circulation during heart repair surgery, for example.

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CHAPTER 6

FUNCTIONAL SIGNIFICANCE OF DIFFERENCES IN MAMMALIAN HEMOGLOBIN AFFINITY FOR OXYGEN

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Introduction

Conventional wisdom, as absorbed by all first year medical students, explains how a decreased hemoglobin affinity for oxygen—a rightward displacement of the oxyhemoglobin dissociation curve (OHDC)—aids the unloading of oxygen in the tissues but compromises loading in the lungs; and vice versa for the case of an increase of hemoglobin affinity for oxygen. A closer look at the participation of hemoglobin in the phenomena of respiratory gas exchange quickly reveals the necessity of also recognizing the effects of the partial pressure of O_2 in the arterial blood, and the arterio-venous oxygen difference, in predicting quantitative influences. Further, the physiological significance acquires a wider perspective if animals with different requirements are considered. The purpose of this brief synopsis will be to examine some of the relevant consequences of different hemoglobin oxygen affinities for gas exchange in normoxic and hypoxic environments; to make a comparative survey in respect to optimality and fitness; and to compare the relative efficacy of adaptations to high altitude that include, on the one hand, an advantageous hemoglobin oxygen affinity, and on the other, a hematopoietic response leading to an increased O_2 carrying capacity.

Range of Mammalian P_{50} and Consequences for Oxygen Exchange

For convenience, the conventional parameter of hemoglobin oxygen affinity, P_{50} (the partial pressure of oxygen at which the hemoglobin is half saturated) will be used subsequently. With this measure Schmidt-Nielsen and Larimer recorded a size-dependent relationship¹⁵ among mammals (although inconsistencies in this trend have been demonstrated⁵, the generality remains valid and useful for discussion). Their graph, shown in Figure 1, reveals a strong tendency for small animals to have hemoglobins with a low affinity for oxygen, and for large animals to have hemoglobins with a high affinity. Remembering that specific metabolic rate is higher in smaller animals, it appears that it is important for them to have hemoglobins that favor oxy-

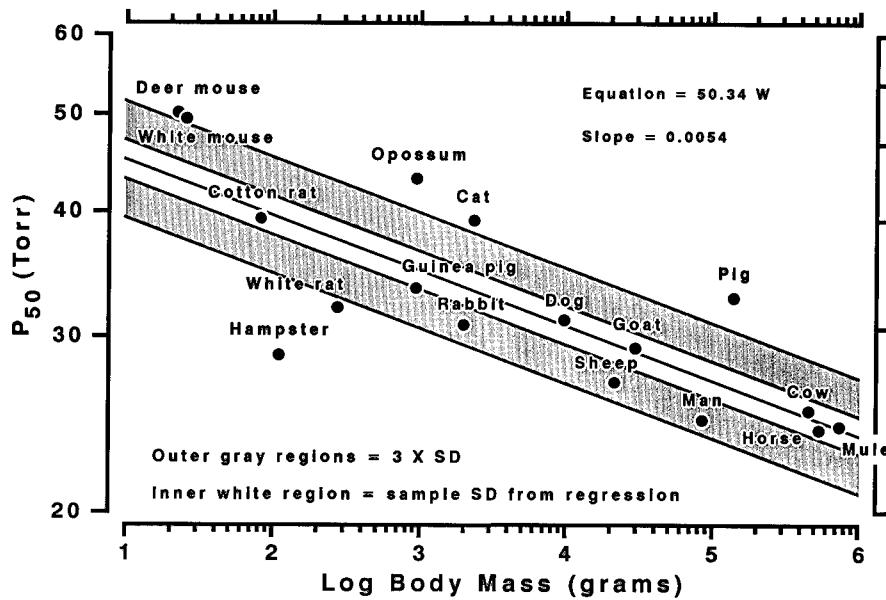


Figure 1 Range of hemoglobin oxygen affinity, expressed as P₅₀, in common mammals, as a function of body size. From Schmidt-Nielsen and Larimer¹⁵, reproduced by permission of the authors and the American Physiological Society.

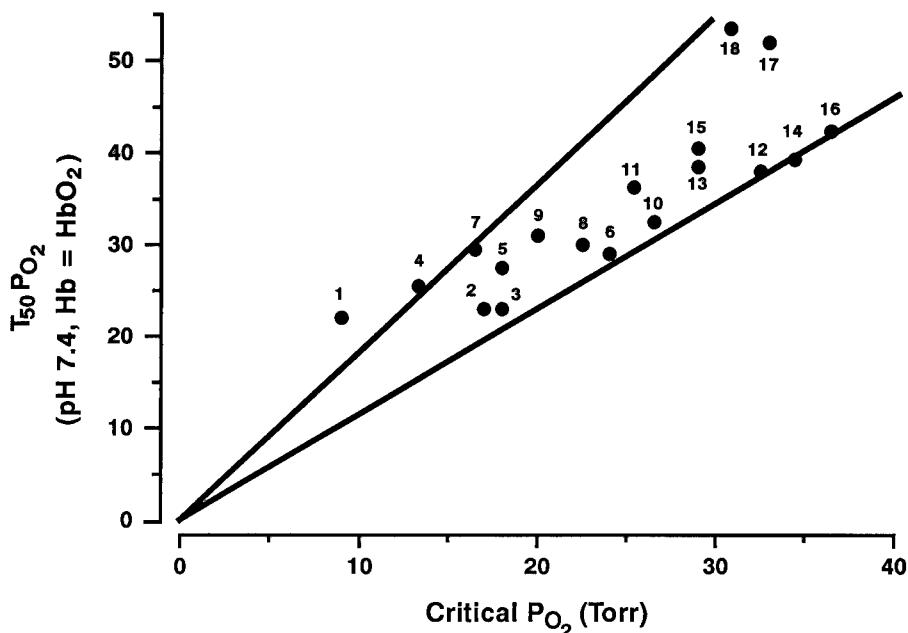


Figure 2 "Critical PO₂" (ambient PO₂ below which oxygen consumption rate cannot be sustained) is higher in those rodents with higher T₅₀ (oxygen tension at which hemoglobin is half saturated). From F.G. Hall¹⁰ reproduced with permission of the American Physiological Society.

gen unloading: the properties of the tissues must have been the dominant factor in natural selection. The advantage of a low P_{50} , defined as the minimal ambient PO_2 that would sustain oxygen consumption, was shown in a series of rodents by F.G. Hall.¹⁰ His graph is reproduced in Figure 2. Survival in hypoxia is apparently favored by a low P_{50} . Hypoxic tolerance is increased. Why hemoglobin oxygen affinity should be high in large animals, generally, is a subject for speculation later, but to favor oxygen loading in the lungs is a definite advantage for those animals that reside at high altitudes.¹¹ In that case, it is likely that environmental pressures would select for a low P_{50} , or, alternatively, that animals with a low P_{50} , would have a competitive advantage for invading high altitude environments and would tend to migrate there.

The range of P_{50} in mammals is roughly from 20 to 50 torr. For reference, human P_{50} is about 30 torr. The effect of P_{50} in gas exchange is dramatized by examining animals selected from the extremes of P_{50} . Figure 3 graphs the OHDC of llama and mouse and shows the arterial and venous points in normoxia for an arterio-venous O_2 saturation difference of 50%. Even in normoxia arterial oxygen saturation is slightly compromised in the mouse, but the venous PO_2 is about 20 torr higher than in the llama. It is also apparent (not shown) that the amount of oxygen that would be unloaded, if the venous PO_2 were the same in the two species, would be much greater in the mouse. In Figure 4 the situation in moderately severe hypoxia, again for a 50% arterio-venous SaO_2 difference, shows a badly handicapped O_2 loading in the mouse,

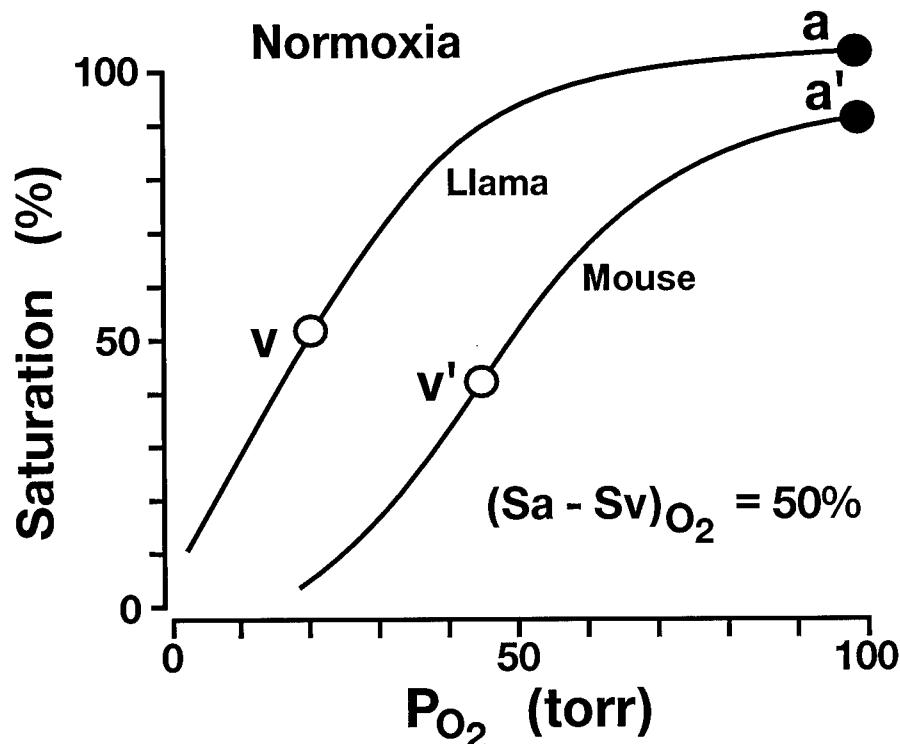


Figure 3 Oxhemoglobin dissociation curves of mouse and llama, plotted in the usual way. These two species were selected to illustrate the extremes of mammalian P_{50} . The arterial and venous points are shown for normoxic condition and an arterio-venous saturation difference of 50%.

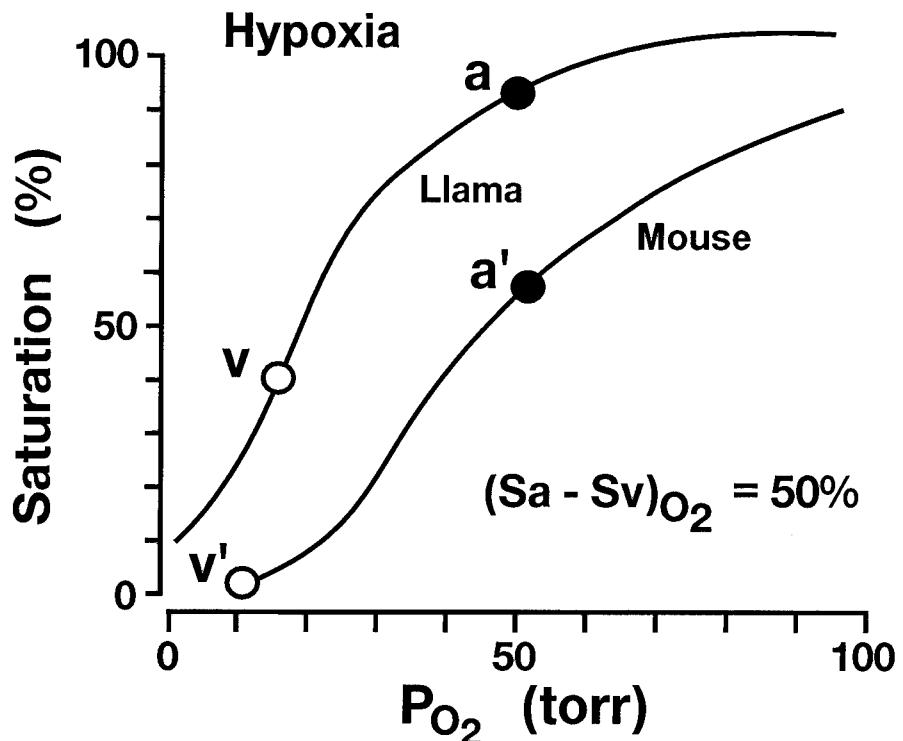


Figure 4 Replica of Figure 3 except that the arterial and venous points are now those that would occur in moderately severe hypoxia.

but now, in contrast to the normoxic condition, the venous PO₂ of the mouse lies slightly below that of the llama. Further, for the same venous PO₂, the llama now has the ability to unload more oxygen. The important fact also emerges, by comparing the normoxic and hypoxic states, that there is a particular arterial PO₂ below which a P₅₀, like that of a mouse, ceases to be of advantage, and a lower P₅₀, like that of a llama, unloads at a higher venous PO₂. Technical jargon sometimes calls this the "cross-over point", but it is intuitively obvious that there must be a particular P₅₀ which will allow maximal oxygen unloading for any given pair of arterial and venous PO₂ values.²²

Optimal P₅₀ for PvO₂ and for (SaO₂ - SvO₂)

Willford, Hill, and Moores²⁴ quantified the problem by expressing mathematically the arterio-venous oxygen saturation difference as a function of the respective partial pressures and then setting the condition of optimality, $d(SaO_2 - SvO_2)/dP_{50} = 0$. This implies $(PaO_2 - PvO_2)^{1/2} = \text{optimum } P_{50}$. In the same manner, but this time maximizing PvO₂ i.e., setting $dPvO_2/dP_{50} = 0$, an optimal P₅₀ can be determined as a function of PaO₂ and (SaO₂ - SvO₂). The consequence of the first set of equations appears graphically in Figure 5 which indicates SaO₂ and SvO₂ as functions of P₅₀. The maximum P₅₀, under these normoxic conditions, is about 50 torr, which approximates that of very small mammals. The shape of the OHDC is also of crucial importance in determining the optimum P₅₀. As P₅₀ increases from 20 torr, the venous blood

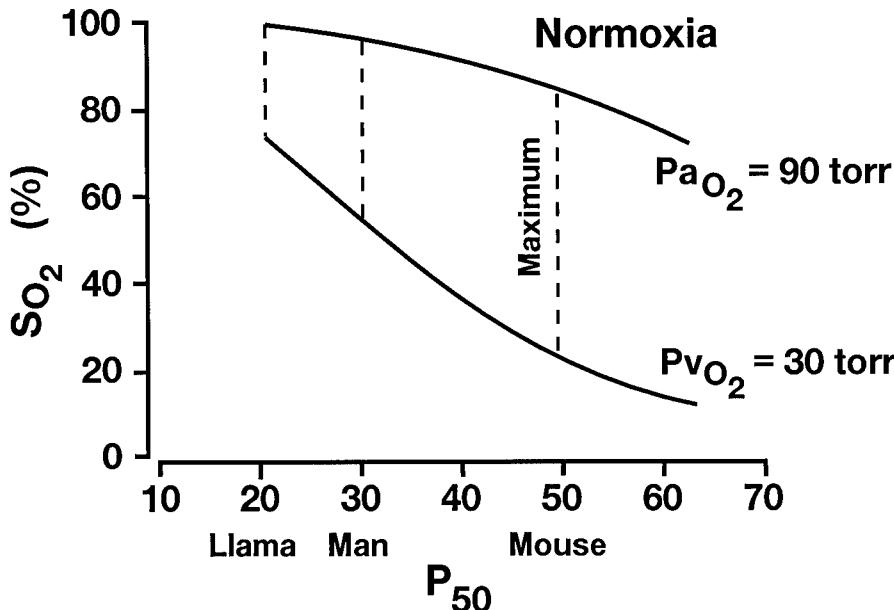


Figure 5 Blood oxygen saturation as a function of P_{50} in normoxia. This kind of representation is adopted to illustrate comparative examples from the more general theoretical analysis of Willford, et al.²⁴

desaturates more rapidly than the arterial blood, up to the point of maximal ($SaO_2 - SvO_2$), but beyond that, it is the other way around. The lower P_{50} in man and llama, as indicated, are far removed from the optimum, and if there were a naturally occurring P_{50} still higher than that of a mouse, less oxygen would be delivered to the tissues in that instance, as well.

When the condition changes to one of hypoxia (Fig. 6) maximum oxygen unloading occurs at a P_{50} of 20 torr, the value found in the llama, and it is the mouse that is farthest removed from the optimum.

Turning now to optimizing venous PO_2 , Figure 7 illustrates the progressive decline of PvO_2 when $P_{50} = 50$ torr and conditions change from normoxia to mild hypoxia. On the other hand, with $P_{50} = 20$ torr, there is a lower venous PO_2 , but it extends over a long plateau as conditions change from normoxia to mild hypoxia. There is however, a "cross-over" when hypoxia becomes more severe. At that critical PaO_2 the venous PO_2 is sustained over a considerable range of further hypoxia, and it is always higher than for blood with a high P_{50} .

The advantage of a low P_{50} at very high altitudes is evident, and native species of mammals—camelids: vicuña, alpaca, llama; rodents: chinchilla, vizcacha—and of birds—huallata, bar-headed goose—fulfill the expectation for fitness in this regard. A study¹⁷ of ten subspecies of deer mice living at different altitudes up to 4000m. found that P_{50} correlated negatively with altitude, although within individual subspecies there was no such correlation (Fig. 8). The evidence therefore points to natural selection for hemoglobins with an optimal P_{50} , and the low P_{50} found in burrowing animals⁹ is consistent with this conclusion. The case of the camelids at high altitude introduces the likelihood of an alternative strategy for optimization. Although high altitude camels have a slightly lower P_{50} than bactrian and dromedary camels at sea

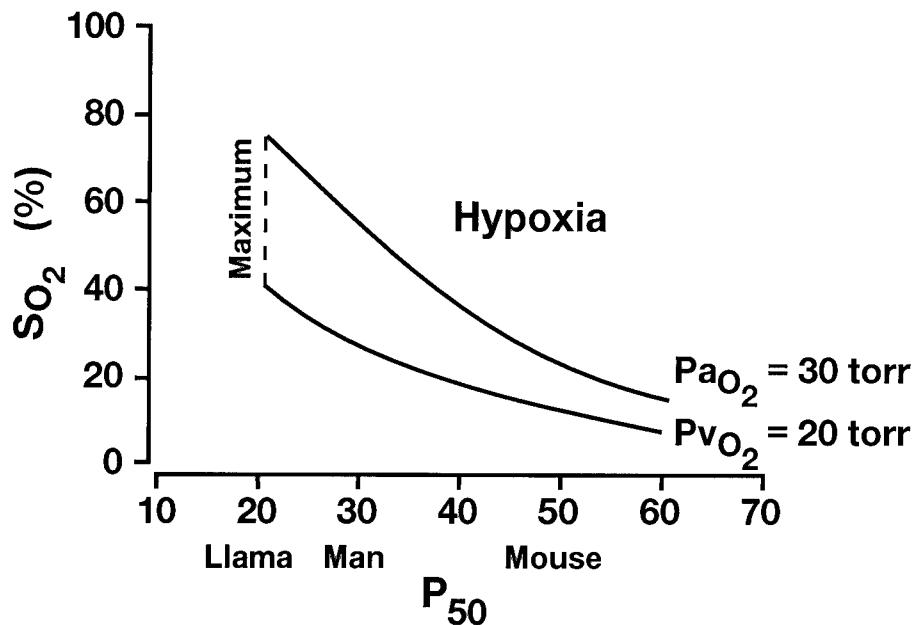


Figure 6 The relationships are the same as for Figure 5, but the condition now is one of hypoxia.

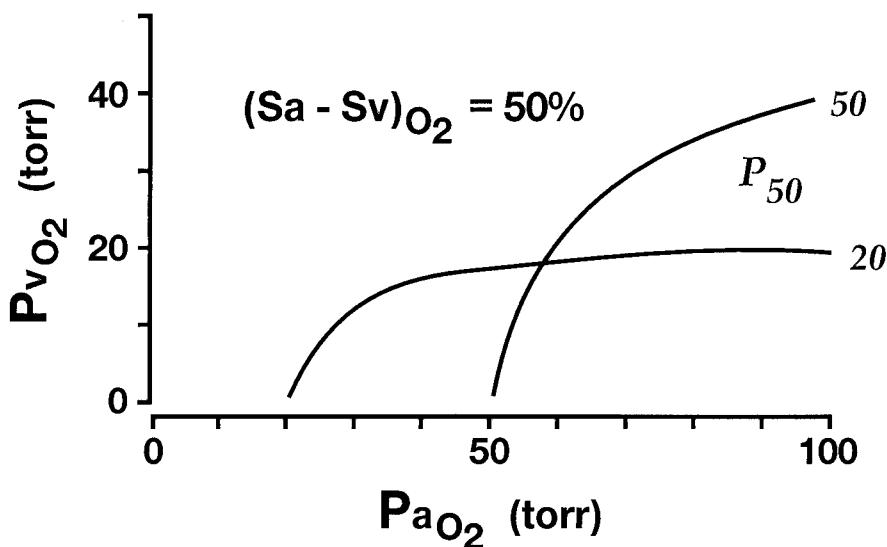


Figure 7 Venous PO_2 as a function of PaO_2 for selected P_{50} 's and a constant arterio-venous difference. This kind of portrayal has been selectively adapted from Willford²⁴.

level, the difference is not great.^{1,6} So, sea level camels had a latent capability which gave them an advantage in oxygen transport in hypoxic environments, and this could have led to an opportunistic migration. More information on the natural history and migration of animals is required to confirm the probable hypothesis that some species

discovered their unique fitness for life in a high altitude environment, and that what the physiologist observes is not, strictly speaking, an adaptation.

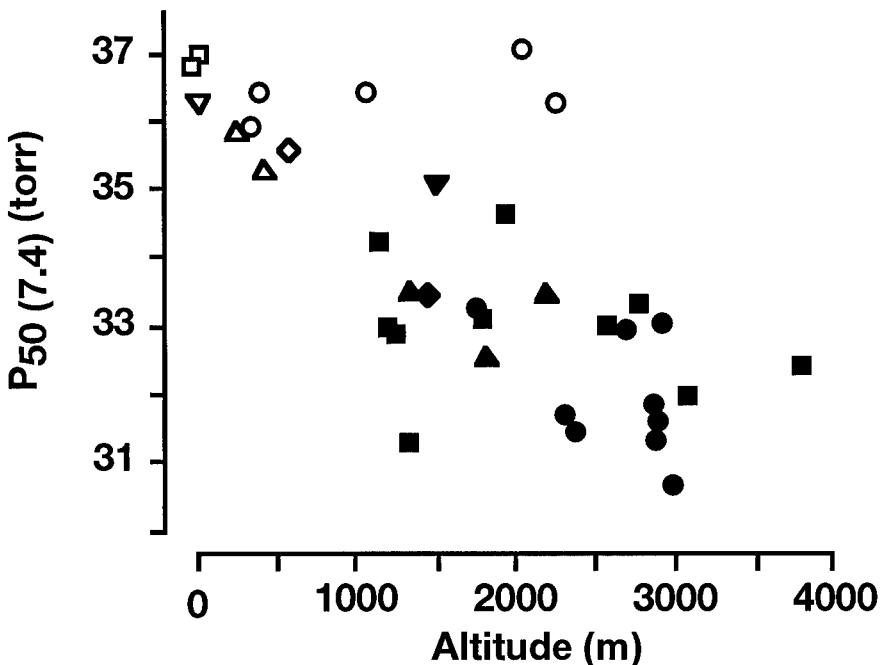
It has sometimes been maintained that the process of acclimatization (not to be confused with adaptation) to high altitude includes some increase in hemoglobin affinity for oxygen, but this appears not to be so.²³ Of course, there is a short-lived shift of the OHDC to the right early in exposure to high altitude due to the acute elevation of intraerythrocytic 2,3 DPG, but that effect is largely overcome by the simultaneous respiratory alkalosis associated with hyperventilation. It is significant that the fine tuning of P_{50} by 2,3 DPG is not a uniform capability in mammals.⁵ Those species with a naturally high P_{50} have very low intraerythrocytic concentrations of 2,3 DPG and their hemoglobins are largely unresponsive to additions of this compound. Species with a low P_{50} have higher concentrations and their hemoglobins react in the expected way (Fig. 9).

Further considerations of optimality

“Efficiency” of O_2 exchange.

The slope of the OHDC (dSO_2/dPO_2) is related to the P_{50} , and it is also a measure of the efficiency of oxygen exchange¹³; this can be an important aspect of the process when oxygen consumption rates and oxygen extraction are high, as in muscular exer-

Blood Oxygen Affinity in Deer Mice



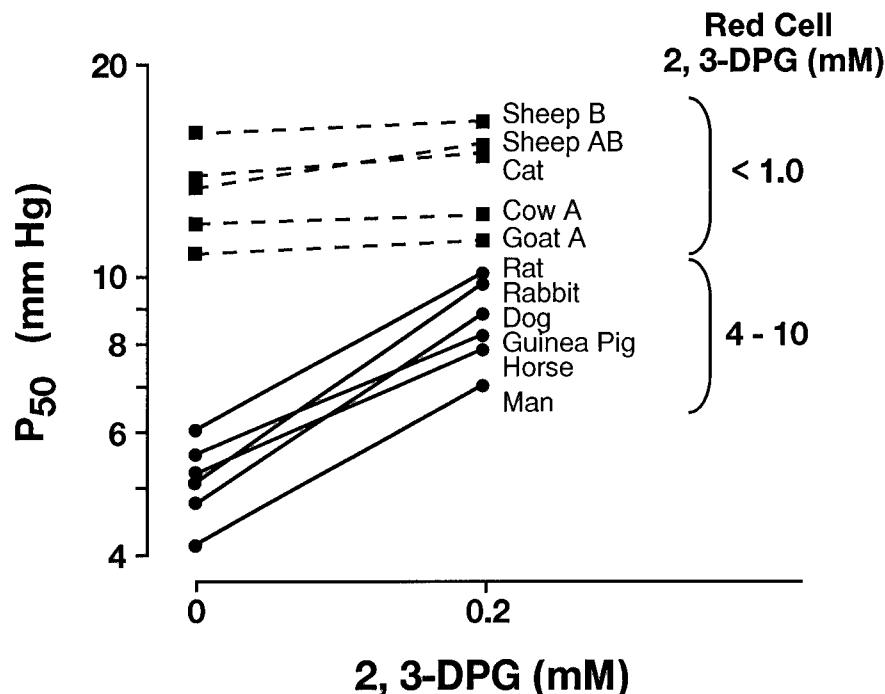


Figure 9 Red cell concentrations of 2,3 DPG in two groups, one characterized by a high P_{50} , the other, low P_{50} , and the reaction isolated hemoglobin solutions of each to added 2,3 DPG. From Bunn⁵, reproduced with permission of author and publisher.

cise. dSO_2/dPO_2 is higher in animals with high hemoglobin oxygen affinity, and it may be this property which is of significance for large animals. Their capacity for heavy exercise, often with impressive endurance, is generally greater than for small animals. Further, mammals² and birds^{3,16} with low P_{50} can reach extremely low venous PO_2 values and still maintain tissue oxygen consumption. The bar-headed goose, for example, almost completely extracts blood oxygen when in flight. There is an, as yet, unexplained feature of the tissues which permits normal function at low PO_2 when the P_{50} is low, but there is apparently an increase of capillary density¹⁸ which would increase the surface area and shorten the distance for diffusion.

Maximum dSO_2/dPO_2 in all hemoglobins is usually found to be located below the P_{50} point. PvO_2 in that region (SO_2 about 38%) is fast approaching the critical value for maintaining aerobic metabolism, and the significance, therefore, of achieving maximum efficiency for exchange is apparent.

Compensatory polycythemia and oxygen transport

A hematopoietic response to hypoxia, and its resultant increase of blood oxygen capacity, is a common phenomenon that is generally interpreted as an important mechanism in acclimatization to high altitude. This response is not significant in animals with a high hemoglobin affinity for oxygen⁶ and, therefore, it is instructive to compare the two cases, one a low P_{50} , and the other, a high hematocrit, as mechanisms for coping with hypoxia.

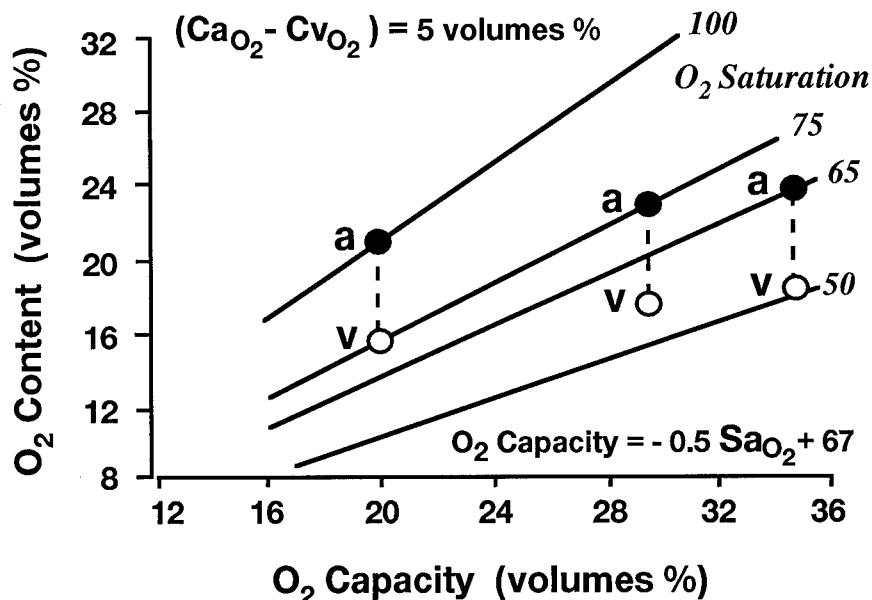


Figure 10 Oxygen content as a function of oxygen capacity and oxygen saturation, the latter shown as a family of straight lines radiating from the origin. The arterial point is determined from the equation shown which describes the hematopoietic response of humans. The arterio-venous content difference remains constant in the three situations shown.

The effect of increasing the oxygen capacity on gas transport in hypoxia is best seen on a plot that relates oxygen content to oxygen capacity as a function of hemoglobin oxygen saturation which is conveniently expressed by a family of straight lines.²¹ The arterial and venous points for a constant arterio-venous O₂ content difference are shown in Figure 10 as increasing hypoxemia develops, and there is the predictable accompanying hematopoietic response leading to increased blood oxygen capacity. The linear equation shown expresses the normal human response which is illustrated. The problem that arises when arterial oxygen saturation is decreasing simultaneously with the increasing oxygen capacity is that the arterial oxygen content rises only slightly but then reaches a maximum and begins to fall in spite of increasing polycythemia. The equation for hematopoietic response, as a function of arterial saturation, becomes an equation for arterial oxygen content by multiplying through by SaO₂. That result is a quadratic equation which can be solved for a maximum by setting its differential equal to zero. Husson and Otis¹² first demonstrated this fact and concluded that the optimum for arterial oxygen content is reached at an arterial oxygen saturation of about 70% and oxygen capacity of about 32 volumes %. This would represent acclimatization to an altitude of about 5500m, the highest, coincidentally, at which permanent human residence has been established. The venous oxygen saturation falls progressively with the diminishing arterial oxygen saturation (and constant arterio-venous oxygen content difference) but less than would be the case if there were no hematopoietic response. In Figure 11 the arterio-venous oxygen content difference widens; in hypoxia the venous oxygen saturation falls from the sea level value of 50% to about 43%. On a standard human OHDC, PvO₂ would have fallen

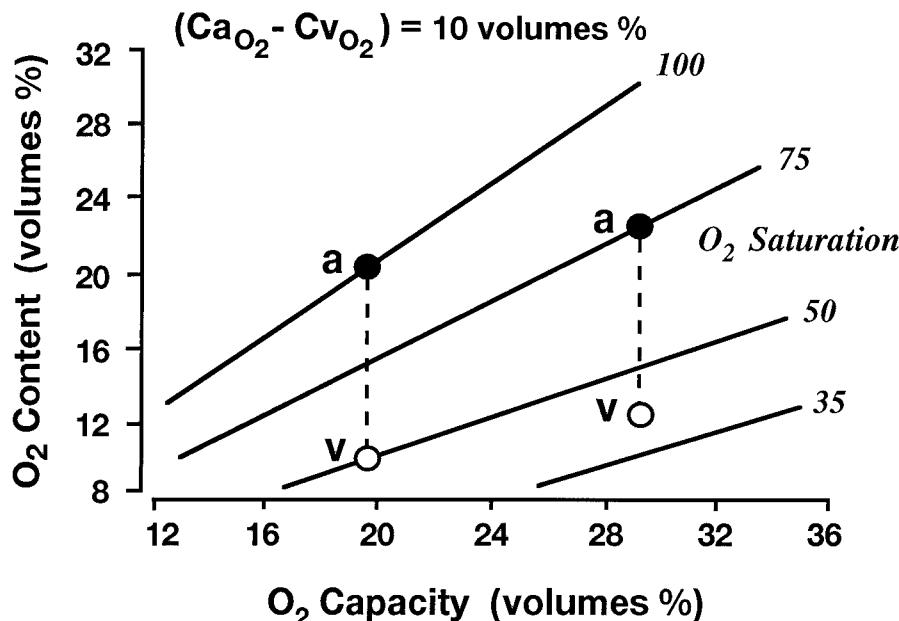


Figure 11 The same as Figure 9 except that the arterio-venous content difference is wider.

from about 30 to 25 torr. The range over which these examples of exchange take place is slightly above the ΔPO_2 where the shape (dSO_2/dPO_2) of the OHDC provides maximum efficiency.

The comparison of relative efficacy of the two cases—one with a low P_{50} and the other with a high O_2 capacity - in severe hypoxia requires consideration of oxygen transport (cardiac output \times CaO_2) in each. In the case of developing hypoxemia and its associated increasing O_2 capacity, CaO_2 was seen (Figs. 10, 11) to rise slightly up to SaO_2 of 70%, but cardiac output (Q) may be compromised by the high blood viscosity. In anesthetized dogs data indicate a steady decline of Q as the hematocrit is altered experimentally from 20 to 75%⁷ which would imply a proportional reduction in oxygen transport in the hypoxic state, since CaO_2 up to $SaO_2 = 70\%$ remains almost constant if the hematopoietic response is normal. However, unanesthetized, acclimatized animals show little or no reduction of Q while resting. Further, high altitude acclimatized animals show an expansion of blood volume¹⁴ which accompanies the rise in hematocrit (Fan's experiments⁷ were isovolumic) and this may be a factor in sustaining the cardiac output. In any case, the increase in blood viscosity must impose an additional burden on cardiac work, a handicap at rest, but a more serious problem in exercise. In fact, the hemodynamic response in exercise may be the crucial consideration. In acclimatized rats exercising at VO_2 (max) Q increased a further 64% when the hematocrit was experimentally reduced from 60 to 45%.⁸

The problems of decreased oxygen transport and increased cardiac work, especially during exercise, with elevated hematocrit, are minimized in animals with a naturally high hemoglobin oxygen affinity. These animals do not increase their hematocrit when hypoxic; in fact, their hematocrit sometimes increases at sea level.⁶ There is also an unexplained negative correlation of total blood volume and P_{50} ³ which can be shown, both interspecifically, and intraspecifically if P_{50} is changed with NaOCN.

The animals with low P_{50} are capable of generating very large increases in cardiac output as well as near maximal oxygen extraction bringing venous PO_2 close to zero, the bar-headed goose⁴ and duck¹⁶ being supreme examples. It is only at the severest degree of hypoxia that the cardiac output begins to wane, probably reflecting a direct myocardial depression.¹⁶

The issue of a minimal tissue oxygen pressure, below which oxidative metabolism cannot be sustained, can be approached through knowledge of venous PO_2 .²⁰ Prediction of interspecific venous values at rest starts with the observation that cardiac output is proportional to resting oxygen consumption rate, therefore, arterio-venous oxygen content difference must be the same in all mammals under basal conditions. Arterial PO_2 is slightly higher in small animals, but the lower P_{50} in large animals tends partially to correct potential differences of arterial oxygen content. If CaO_2 were a constant, then so too would be CvO_2 , but now the high P_{50} of small animals will lead to a higher PvO_2 in those species. Data on this point are limited, but what are available do not indicate a significant difference between large and small animals. However, when oxygen consumption rate rises in exercise and oxygen extraction increases, the small animals lose their advantage, because the efficiency of unloading is less than in large animals as discussed above.

Summary

Small mammals, generally, have hemoglobins with a low O_2 -affinity (high P_{50}) which favors O_2 -unloading in the tissues and may be regarded as an adaptation suited to their high specific metabolic rate. Large animals, generally, have hemoglobins with a high O_2 -affinity suitably adapted for life in hypoxic environments but also of advantage in heavy exercise.

Optimizing O_2 extraction and maximizing venous PO_2 are functions of P_{50} . Individual species have evolved with P_{50} 's near the optimum for their habitat.

A comparison of the two strategies in the blood for adapting to high altitude, hematopoietic response with resultant increase of oxygen capacity or hemoglobin with a high oxygen affinity, suggests that the latter is the more favorable. A high hematocrit in the hematopoietic strategy leads to increased blood viscosity and increased cardiac work, even if cardiac output is maintained at rest, but in exercise the cardiac output response is below normal, and, therefore, oxygen transport is compromised. Animals with a low P_{50} have normal, or even low, hematocrits, are not handicapped by a high blood viscosity and are capable of achieving very high cardiac outputs and high rates of convective oxygen transport in exercise.

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CHAPTER 7

HYPOXIC VENTILATORY CONTROL AND HEMOGLOBIN OXYGEN AFFINITY

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Introduction

The arterial partial pressure of oxygen at which a ventilatory response is initiated, known as the "hypoxic ventilatory threshold" (HVT), varies considerably between species. Comparative studies suggest a number of correlates with these differences in hypoxic sensitivity including size and adaptations to hypoxic habitats, but a common and perhaps mechanistically more proximate co-variant is the affinity of the animal's hemoglobin for oxygen. The patterns of interspecific and intraspecific variation in hypoxic ventilatory threshold will be reviewed to determine how they may or may not relate to the characteristics of the animal's hemoglobin, including quantity and affinity, and how that in turn may relate to the role of heme-proteins in oxygen-sensing mechanisms within the carotid body type-I cells.

Interspecific Comparisons

Boggs and Tenney¹² described a relationship between the ventilatory response to 12% inspired oxygen and body size that had a slope of -0.25, using mammals whose body masses varied from 0.04kg to 400kg. Smaller mammals exhibited a greater increase in ventilation than large ones at the same level of inspired oxygen. This seemed reasonable insofar as the smaller mammals have a higher mass-specific metabolism and hence a need to deliver oxygen to their tissues at a proportionately higher rate. If the receptors responsible for driving ventilation do so in response to the ratio between the rate of oxygen delivery to the tissues and the rate of oxygen consumption by those tissues, and the subsequent PvO_2 , then species with higher metabolic rates per gram of tissue should have a more brisk ventilatory response to any given level of hypoxia. It should be noted, however, that this inverse relationship between size and hypoxic response was not confirmed in another study by Frappell *et al*¹⁸ who suggested that the response to 10% oxygen was roughly equivalent in marsupial and eutherian mammals 0.008-50 kg. This issue is confused by the fact that small mammals tend to reduce their metabolic rate in response to hypoxia and therefore one really must use convection requirement (the ratio of ventilation to metabolic rate) as the index of response rather than the ventilation alone, and if one does that then there is at least some indication that smaller mammals have a greater response than large ones to a given level of inspired oxygen in the Frappell *et al* study as well.

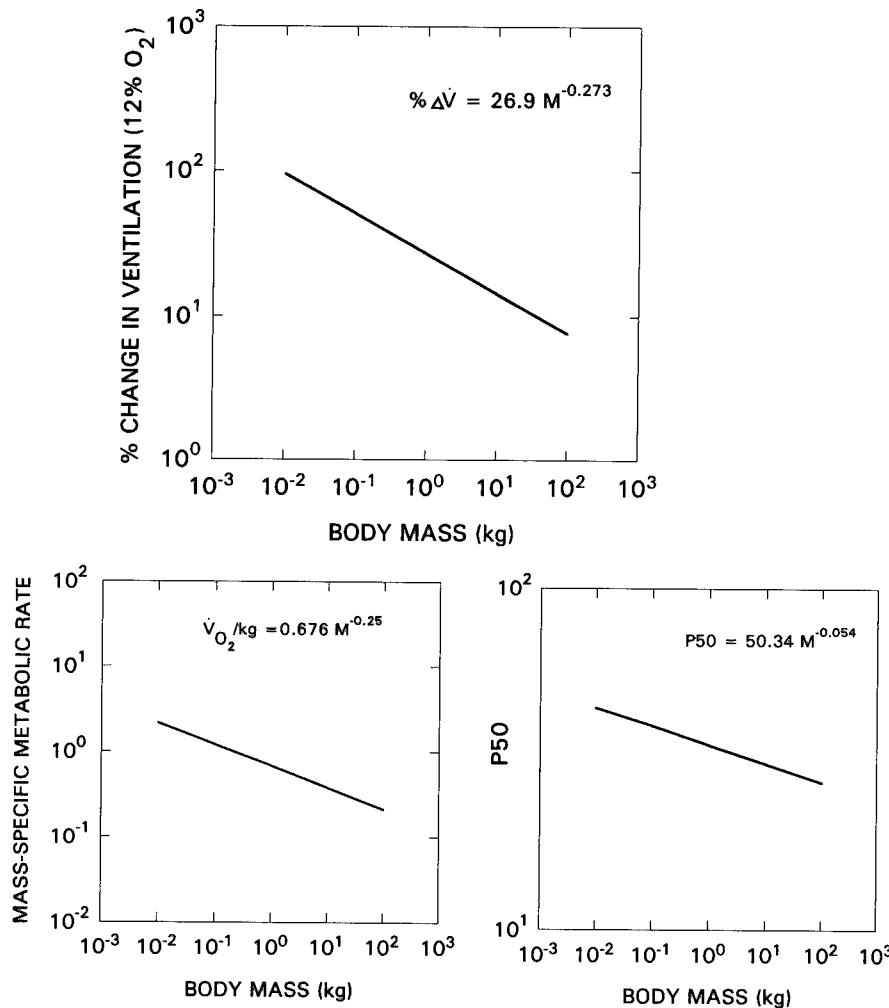


Figure 1 A. Relationship between ventilatory response to 12% O₂ and body mass, redrawn from 12. B. Relationship between mass-specific metabolic rate and body mass, redrawn from equation in ref.43. C. Relationship between hemoglobin P50 and body mass, redrawn from equation in ref 42.

In any case, if we assume for the moment that there is an inverse relationship between size and hypoxic ventilatory response (Fig. 1a; Ref. 12), this could reflect either the similar relationship between size and mass-specific metabolic rate (Fig. 1b; Ref. 43), or the relationship between size and the affinity of hemoglobin for oxygen (Fig. 1c), wherein the hemoglobin of smaller mammals tends to have a lower affinity for oxygen than that of the larger mammals⁴². Walsh⁴⁶ recently studied the small (40g) lesser spear-nosed bat, *Phyllostomus discolor*, as a convenient subject to distinguish between those alternatives—i.e. do smaller mammals have a brisker hypoxic response because they have a higher mass-specific metabolic rate or because they have a lower affinity hemoglobin. This bat is unusual in having quite a high affinity hemoglobin in spite of its small size and high mass-specific metabolic rate. The response of the

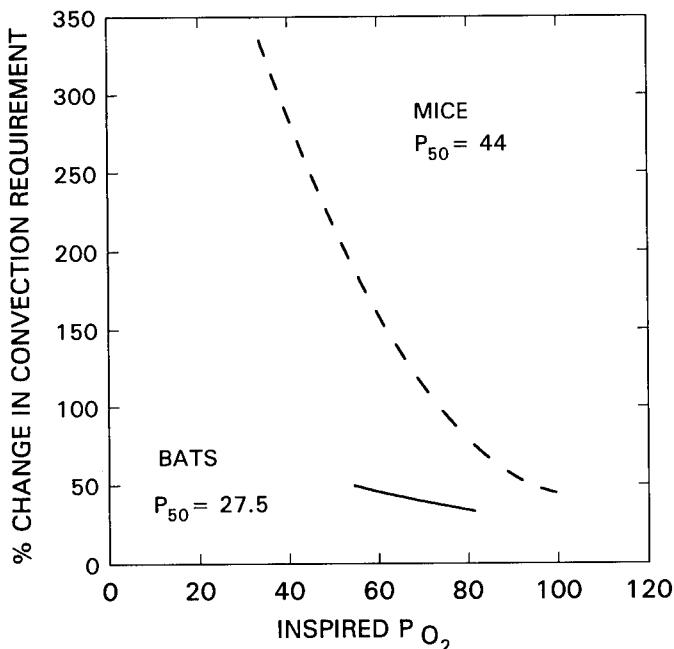


Figure 2 Percent change in convection requirement with varying inspired partial pressure of oxygen in lesser spear-nosed bats (solid line) compared to laboratory mice (dashed line). Redrawn from 46.

lesser spear-nosed bat to hypoxia was compared to that of a similar sized laboratory mouse who has a lower affinity hemoglobin. If the mechanistic link between size and hypoxic response is mass-specific metabolic rate then they should have similar responses, but if the more appropriate link is hemoglobin affinity, then the bat should have a reduced response compared to the mouse. The bat with the lower P50, higher affinity hemoglobin, exhibits a lower threshold to its hypoxic response (expressed as % change in convection requirement to take account of the hypoxic hypometabolism of these species) than the mouse with its higher P50, lower affinity hemoglobin (Fig. 2).

Examples of this phenomenon are repeated in comparisons between species of the same size who have differing hemoglobin affinities due to adaptations to hypoxic environments whether they be fossorial or high altitude environments. Arieli and Ari³ found that the fossorial mole rat has a lower threshold to its hypoxic ventilatory response than the white rat of similar body size and has a correspondingly lower P50 (Fig. 3). Figure 4, redrawn from van Nice *et al.*⁴⁵ demonstrates the same phenomenon for three other mammals, including the high altitude-adapted llama.

John Weil *et al.*⁵⁰ noted long ago in humans that the hypoxic ventilatory response curve corresponds quite well to an inverted hemoglobin saturation curve wherein the range of PaO_2 over which the hemoglobin begins to desaturate corresponds to the range of PaO_2 over which the ventilation begins to increase. This seems to be the case for other mammals as well and the same general trends can be seen in birds in Fig. 5. The rhea¹⁰ and bar-headed goose⁹ have higher affinity hemoglobins and associated lower hypoxic ventilatory thresholds when compared to the pheasant¹⁰ or the burrowing owl (Kilgore, unpublished). The degree of desaturation of the hemoglobin that

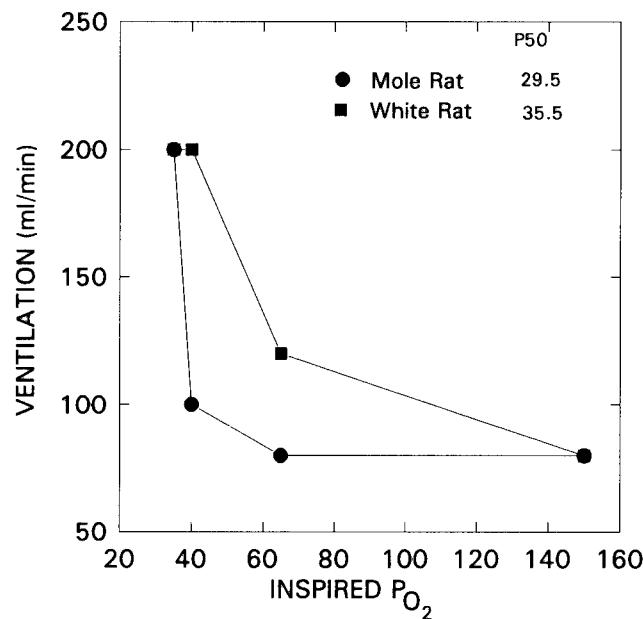


Figure 3 Ventilation in response to declining inspired oxygen levels in the mole rat (circles) with a high affinity hemoglobin compared to the laboratory white rat (squares) with a lower affinity hemoglobin. Redrawn from 4 and 5.

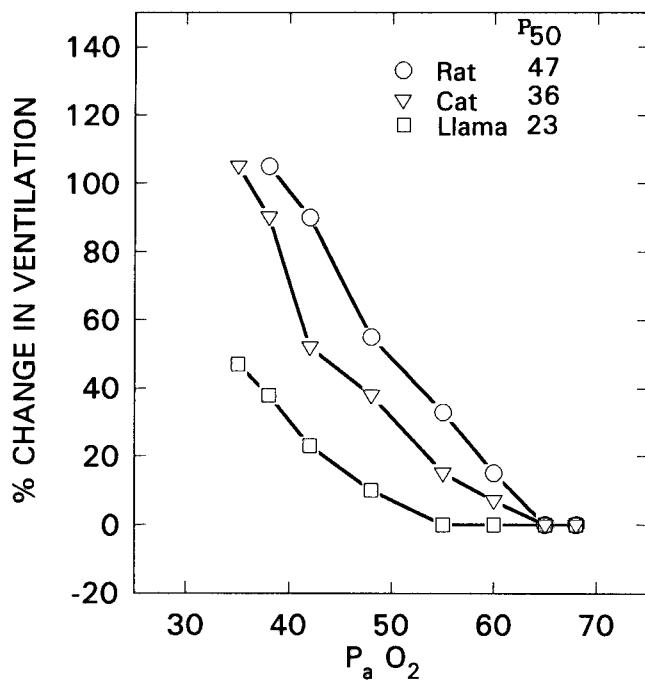


Figure 4 Percent change in ventilation as a function of arterial partial pressure of oxygen in the rat (circles), cat (triangles), llama (squares) (from 45).

corresponds to the HVT varies considerably between species and depends upon how the HVT is defined (i.e. how much of a change in ventilation is considered to represent the threshold) and whether the hypoxic response is isocapnic or hypocapnic. The HVT is usually defined as the highest PaO_2 at which a statistically significant change in ventilation occurs. A change in ventilation in excess of 10% was used as the HVT to generate the values from literature data presented in Table 1 and Fig. 10. HVT ranges from 75-95% saturation and tends among mammals to be higher for those with a higher affinity hemoglobin (human, llama, ground squirrel). The woodchuck, porcupine and birds HVT is at lower % saturation but they were all studied under hypocapnic (or poikilocapnic) hypoxic conditions and birds as a group may be different in this regard. The dissociation curves of high affinity hemoglobins tend to be very steep over their desaturating range compared to those of lower affinity hemoglobins. Van Nice *et al*⁴⁵ point out that the slopes of the ventilatory responses plotted against saturation also tend to be much steeper for those species with higher affinity hemoglobins.

Table 1.

Species	%Sat at HVT	P50	Reference
Rat	75-85%	47	(7)
Cat	86%	36	(45)
Llama	92%	23	(45)
Ground Squirrel	92%	18	(37)
Woodchuck and Porcupine	70-75%	28 31	(11)
Human(isocapnic)	95%	27	(50)
Pheasant	85%	42.4	(10)
Rhea	80%	30.5	(10)
Bar-headed Goose	60%	27.2	(9)
Burrowing Owl	80%	42.3	Kilgore

Intraspecific Comparisons

What kinds of relationships can be seen intraspecifically between hemoglobin characteristics and hypoxic ventilatory response? Many studies have addressed the question of whether it is oxygen content of blood at any given PaO_2 that is important in stimulating ventilation or only the partial pressure of oxygen in arterial blood. Reducing the amount of hemoglobin by inducing various levels of anemia has been shown in goats⁴¹ and rats⁶ to have little effect on ventilation but substantial effects upon cardiac output. This has been demonstrated in other tetrapod groups as well. Among birds, ducks have been shown to exhibit no ventilatory response to declining hematocrit but a substantial increase in heart rate (Boggs and Tenney, unpublished)

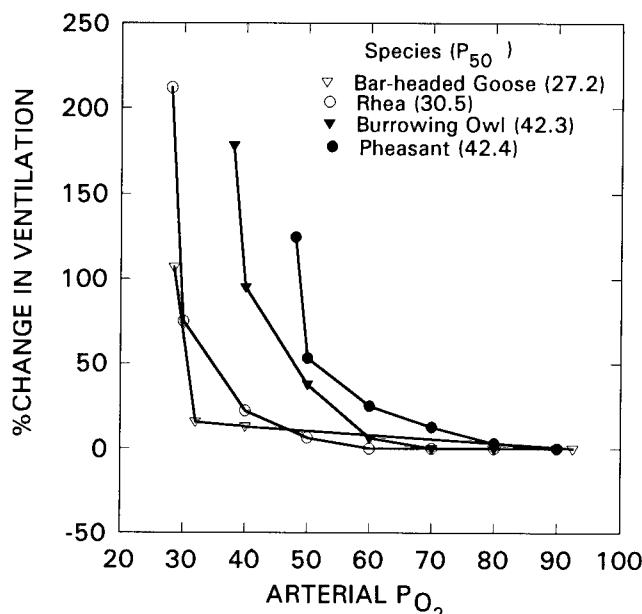


Figure 5 Percent change in ventilation as a function of arterial partial pressure of oxygen in Bar-headed geese (open triangles) from 9; rhea (open circles) from 10; burrowing owl (solid triangles) from Kilgore, unpublished; and pheasant (solid dots) from 10.

(Fig. 6). A similar pattern has recently been demonstrated in an amphibian, the toad, by Tobias Wang *et al*⁴⁷.

Changing oxygen content without changing hematocrit by binding carbon-monoxide to hemoglobin tends to stimulate aortic chemoreceptors far more than carotid chemoreceptors^{33,25} and cardiovascular more than ventilatory responses. It has been suggested that the very high blood flow in the carotid bodies makes them relatively insensitive to changes in oxygen content but quite sensitive to changes in partial pressure of oxygen in the perfusate (be it plasma or whole blood). There are reports however of significant ventilatory responses to the fall in oxygen content associated with carboxyhemoglobinemia in cats^{21,22} and more recently in golden-mantled ground squirrels²⁰.

Although there appears to be a reasonably consistent correlation between hypoxic ventilatory threshold and hemoglobin affinity interspecifically a less consistent picture emerges from intraspecific studies of the effects of varying hemoglobin affinity on hypoxic ventilatory response. Birchard and Tenney⁷ increased the affinity of hemoglobin in a test group of rats by treating them with sodium cyanate for three weeks. The hypoxic ventilatory responses of the rats with artificially elevated hemoglobin affinity did not differ significantly from that of control rats (Fig. 7). A similar lack of effect of varying Hb affinity on hypoxic response was observed in goats in a study by Santiago and Edelman⁴¹ wherein the change in P50 occurred in response to chronic anemia.

Genetic variations in hemoglobin affinity can also be used to assess the intraspecific affects of affinity on hypoxic ventilatory response. In humans a genetic variation in hemoglobin affinity is represented by the high affinity Andrews-Minneapolis Hb (with a β chain mutation). A study of members of the same family with and without

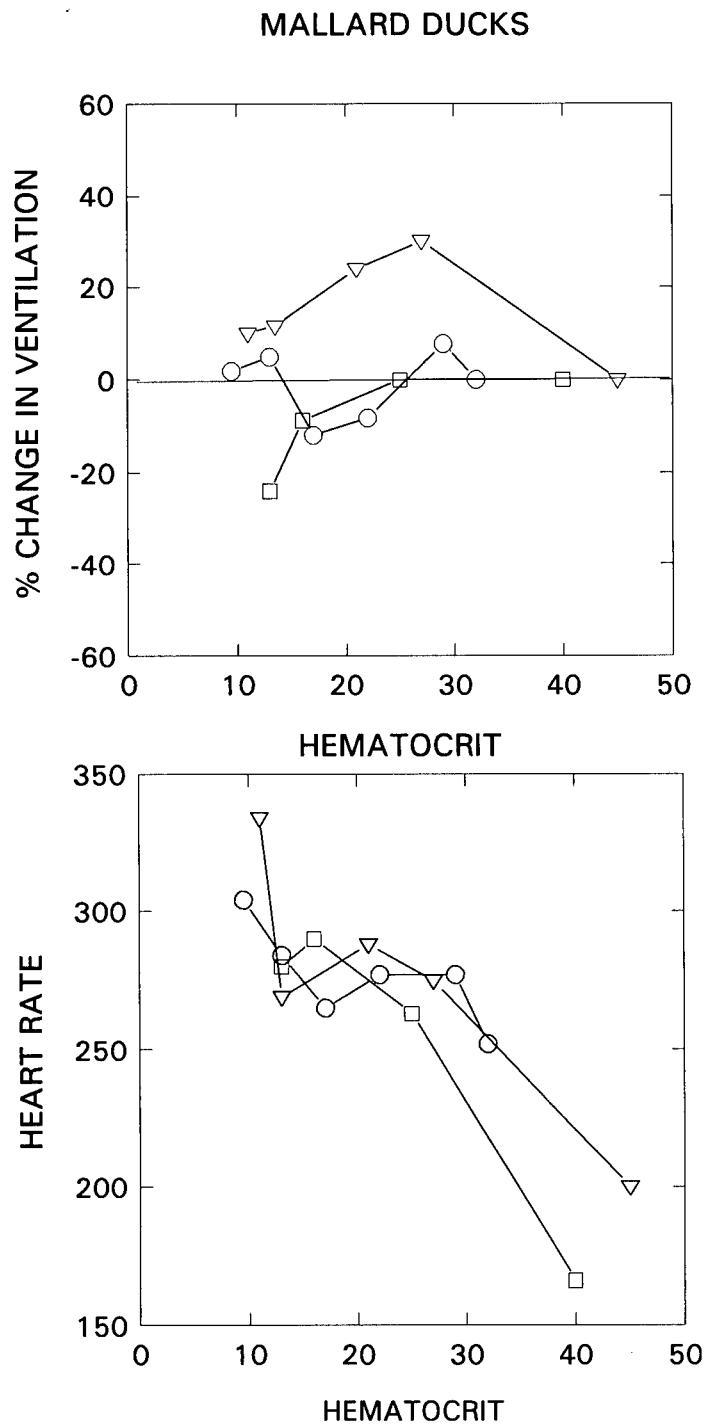


Figure 6 Effect of declining hematocrit on heart rate and ventilation in three mallard ducks (from Boggs and Tenney, unpublished).

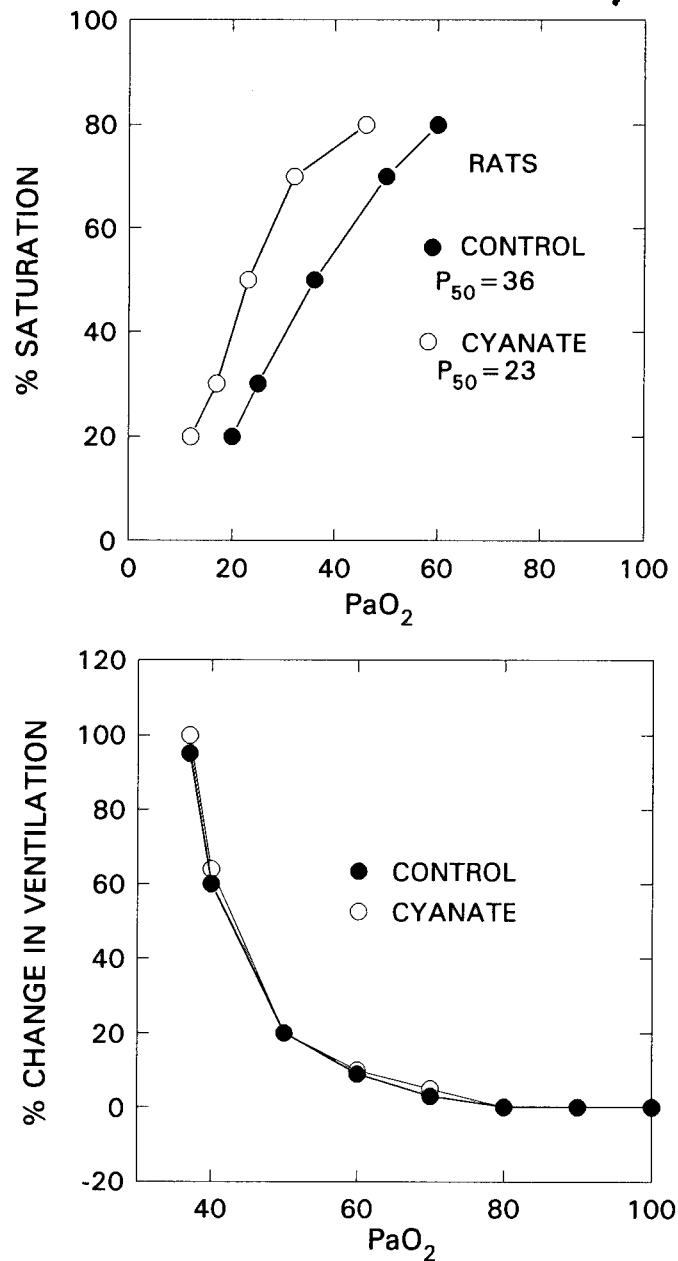


Figure 7 A. Effect of chronic sodium cyanate treatment upon hemoglobin oxygen affinity in rats. B. Comparison of ventilatory response to hypoxia in sodium cyanate treated (open circles) and control (solid dots) animals illustrating no difference in hypoxic ventilatory response in spite of differences in hemoglobin affinity. Redrawn from 7.

this type of hemoglobin revealed no difference in hypoxic response²⁷. On the other hand genetically distinct hemoglobins of varying affinity are found to occur naturally

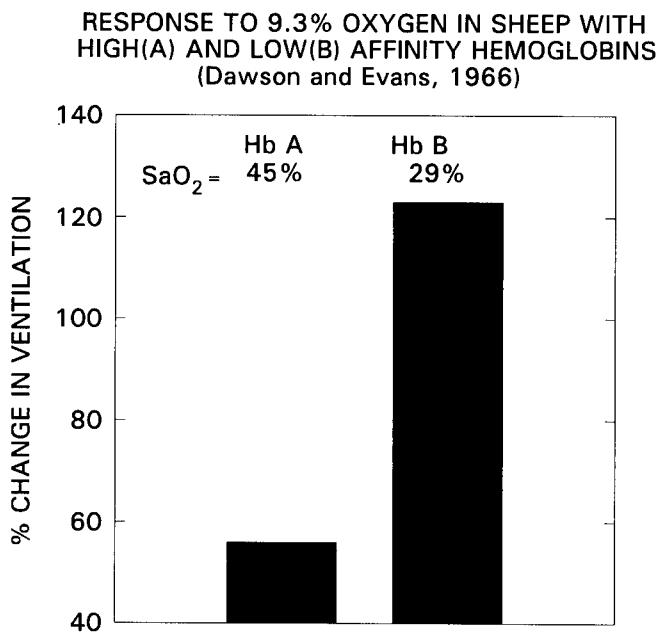


Figure 8 Comparison of the ventilatory response to 9.3 % oxygen in sheep with high affinity hemoglobin (A) and normal hemoglobin affinity (B) indicating a relatively reduced ventilatory response to hypoxia in the sheep with a high affinity hemoglobin. Data from 15.

among sheep, and in this case there is a correspondence between a higher affinity hemoglobin and a lower hypoxic ventilatory threshold¹⁵ (Fig. 8).

Another naturally occurring cause of variation in hemoglobin affinity intraspecifically and within an individual is changing temperature. The effects of changing body temperature on both hemoglobin affinity and hypoxic ventilatory response can be observed in poikilothermic endotherms such as hibernating mammals and in poikilothermic ectotherms such as reptiles. Maginniss and Milsom³⁵ have shown both a temperature dependent and a temperature-independent hibernation effect upon the hemoglobin affinity of golden-mantled ground squirrels. The hibernation effect increases the affinity of the hemoglobin through decreased concentrations of organic phosphates and increases the amount of hemoglobin, while the decreased temperature during hibernation also induces a large shift of the curve to the left. A substantial temperature dependent increase in hemoglobin affinity has also been described for hibernating hedgehogs¹³ and thirteen-lined ground squirrels³⁹. Associated with the low body temperature and the increased hemoglobin affinity during hibernation is a change in the hypoxic ventilatory response of golden-mantled and columbian ground squirrels as demonstrated by McArthur and Milsom³⁶ (Fig. 9).

Reptiles exhibit a similar pattern relating changing hemoglobin affinity intraspecifically or individually with changing temperature to shifts in the hypoxic ventilatory response. Glass *et al*²³ show this beautifully in the freshwater turtle, *Chrysemys picta bellii*, (Fig. 9). A similar pattern is seen in the Mexican black iguana¹⁷ and another turtle *Pseudemys scripta*²⁹. The pattern is qualitatively the same as observed in mammals with temperature induced changes in affinity or with interspecific

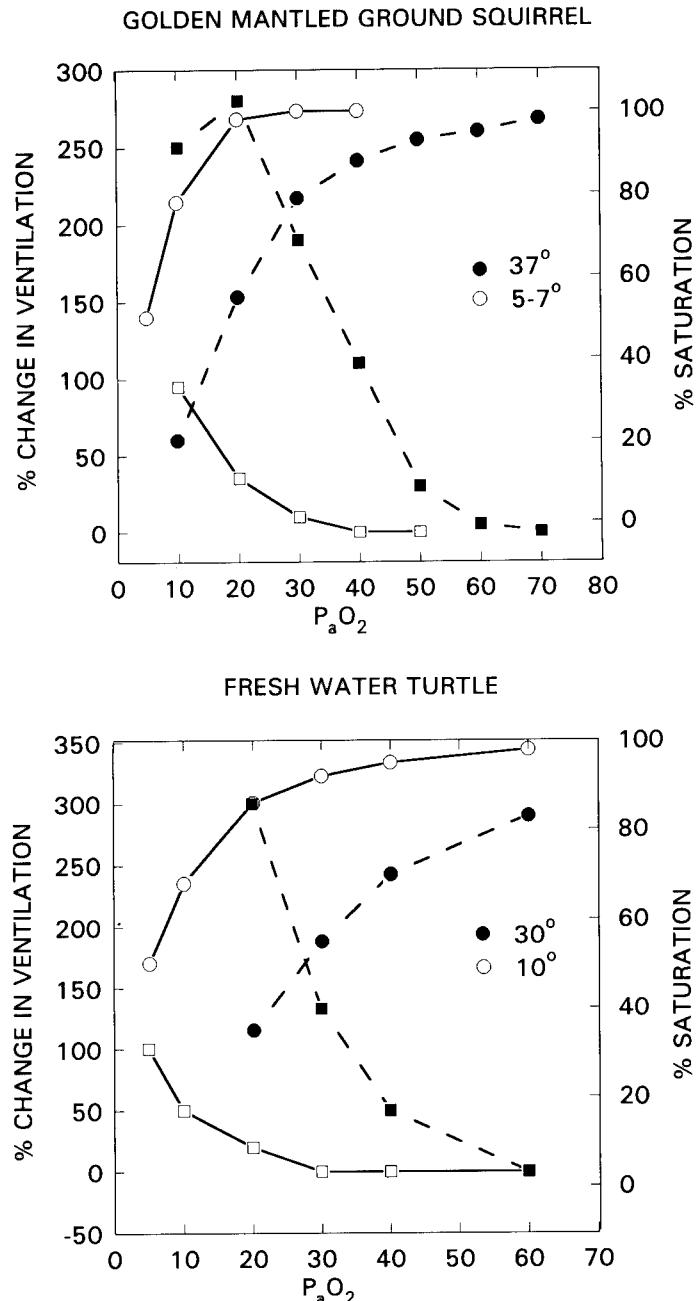


Figure 9 A. Percent change in ventilation in golden mantled ground squirrels, *Spermophilus columbianus*, with change in arterial partial pressure of oxygen at two different temperatures (solid symbols, 37°C; open symbols 5° for ventilation and 7° for hemoglobin equilibrium curve) and two different hemoglobin affinities demonstrating the way in which they shift together. Data from 35,36. B. Percent change in ventilation and percent hemoglobin saturation in the fresh water turtle, *Chrysemys picta bellii*, as a function of arterial partial pressure of oxygen at 30° (solid symbols) and 10° C (open circles). Data from 23.

differences in affinity except that in mammals the hypoxic threshold tends to correspond to a higher level of saturation than is seen in reptiles whose normal arterial saturation is lower due to cardiac shunts.

Figure 10 summarizes some of the data for several species of birds, mammals, and reptiles on HVT and P50. There is clear trend for HVT to increase as P50 increases. The considerable scatter in the data for birds and mammals reflects the variability between studies in such important confounding factors as measurement techniques and concomitant hypocapnia which would shift the HVT to lower PaO_2 s and may do so to different extents in different species. A systematic comparative study using consistent techniques and isocapnic hypoxia is needed and may reveal a tighter relationship. Nevertheless there seems to be a clear correlation in broad interspecific comparisons between HVT and P50.

O_2 -sensors In The Control of Breathing

We have seen inter- and intra- specifically a parsimonious pattern to 'match' the hypoxic ventilatory response to hemoglobin affinity so as to avoid, on the one hand, the work of increasing ventilation before declining partial pressures of oxygen have caused significant desaturation of the hemoglobin, but to insure, on the other hand, that ventilation is stimulated when hypoxia does become sufficient to compromise saturation. The next obvious question to ask is, how does that pattern arise? What mechanistic connection could there be between hemoglobin affinity and the hypoxic ventilatory response? To address this we need to review the current understanding of the transduction processes in oxygen sensors in general, and in the carotid bodies in particular.

One potential connection may reside in the fact that cellular oxygen sensing mechanisms in general appear to involve a heme-protein distinct from mitochondrial cytochromes and of a much lower oxygen affinity. Such heme-proteins are associated with enzymes such as NAD(P)H oxidases, catalases, guanylate cyclases, or with membrane ion channels, and have been described in or ascribed to several oxygen-sensing cell types including erythropoietin producing hepatoma cells^{26,24}, neuroepithelial bodies in the lungs⁵², smooth muscle cells in the pulmonary vasculature⁴⁰ and carotid body type I cells^{1,14,31,33} (Table 2).

A variety of models are currently being explored to describe the specific roles of these heme proteins at each of these sites. In neutrophils the NADPH oxidase produces oxygen radicals and H_2O_2 to destroy phagocytized microbes. As Acker³ describes the model for erythropoietic cells, the heme protein is or is associated with an NAD(P)H-oxidase that forms hydrogen peroxide (H_2O_2) which in this case is used as a second messenger. A decline in tissue partial pressure of oxygen would lead to reduced amounts of H_2O_2 which in turn affects transcriptional factors that induce the production of erythropoietin. In the carotid bodies the type I (or glomus) cells are the transducers that sense changes in partial pressure of oxygen and convert that signal into increased discharge frequency in the afferent nerve by releasing dopamine as a neurotransmitter substance across synaptic contacts with afferent nerve endings. The link between declining PaO_2 and release of neurotransmitter from type I cells may also involve an NAD(P)H oxidase heme protein, H_2O_2 , and/or other intermediaries such as cGMP or cAMP which in turn alter the open-probability of membrane K^+ channels³³. Or it may be simpler than that. The simplest model places the heme-protein O_2 -sensor in the cell membrane in contact with or even as a part of the K^+ -chan-

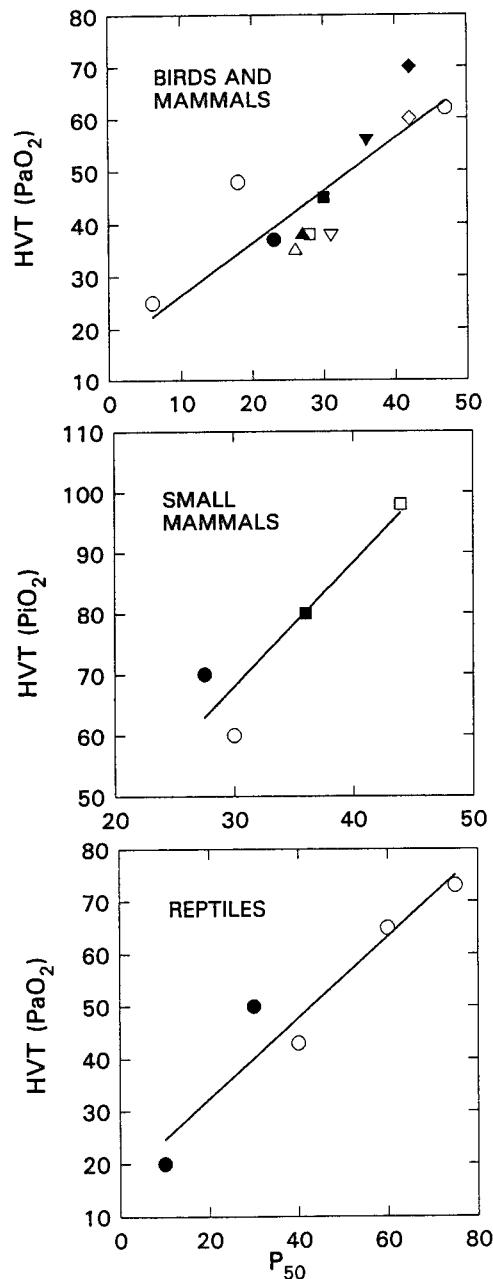


Figure 10 Summary of the relationship between P_{50} and HVT (Hypoxic Ventilatory Threshold) a: expressed in terms of PaO_2 for several mammals and birds - ground squirrel (open circle)³⁶, llama (filled circle)⁴⁵, human (open triangle)³⁸, barheaded geese (filled triangle)⁹, woodchuck (open square)¹¹, rhea (filled square)¹⁰, porcupine (open inverted triangle)¹¹, cat (solid inverted triangle)⁴⁵, burrowing owl (open diamond) (Kilgore, unpublished), pheasant (filled diamond)¹⁰ and rat (dotted open circle)⁷. b. HVT expressed in terms of inspired partial pressure of oxygen for the small mammals—bat (filled circle)⁴⁶, mole-rat (open circle)⁵, rat (filled square)⁵, and mouse (open square)⁴⁵. c. HVT of the fresh water turtle (filled circles)²³ and black iguana (open circles)¹⁷ as P_{50} changes with temperature.

nel. Patch-clamp studies of isolated membrane K⁺-channels show a maintenance of the Po₂-dependence of the K⁺ current¹⁹ suggesting that intracellular second messengers are not a necessary link between Po₂ and the state of K⁺ channels. Figure 11 (redrawn from Ref. 25) depicts the simplest model of carotid type I cell transduction. When tissue Po₂ is high enough the membrane-bound heme protein associated with K⁺ channels binds O₂ and in that configuration the 'open-probability' of the K⁺ channel is high. When tissue Po₂ falls the membrane bound heme protein will become desaturated and the resulting conformation change would reduce the probability that the K⁺ channel would be open. The reduced outward K⁺-current would depolarize the type I cell membrane which would open voltage-gated Ca⁺⁺ channels. The Ca⁺⁺ influx would cause the release of transmitter from the synaptic vesicles and thus increase discharge frequency from the carotid sinus nerve stimulating the central respiratory control centers to increase ventilation. The transduction mechanism may be more complex than this, involving other simultaneous intracellular pathways, since the magnitude of the neural response to K⁺-channel blockers is not as great as that induced by hypoxia¹⁶.

Table 2.**CELLULAR O₂-SENSING SYSTEMS**

ERYTHROPOIETIC CELLS	NADPH OXIDASE (b type cytochrome) (24,26)
NEUROEPITHELIAL CELLS	NADPH OXIDASE (b type cytochrome) O ₂ -DEPENDENT K ⁺ CHANNELS IN PLASMA MEMBRANE (52)
CAROTID BODY TYPE I CELLS	NADPH OXIDASE (b type cytochrome) O ₂ -DEPENDENT K ⁺ CHANNELS IN PLASMA MEMBRANE (3, 33,34,25)
PULMONARY SMOOTH MUSCLE CELLS	O ₂ -DEPENDENT K ⁺ CHANNELS (40)

Other mechanisms for the hypoxic response have been suggested that involve a lower affinity cytochrome aa₃ in type I cell mitochondria than in the mitochondria of other cell types. Hypoxia would retard electron transfer through the electron transfer chain resulting in a decreased proton electrochemical gradient which would lead to the release of intramitochondrial Ca⁺⁺ as the stimulus for transmitter release⁸. However it has since become apparent that the mitochondria do not represent an adequate source of Ca⁺⁺. Lahiri³¹ has recently suggested a dual mechanism whereby the low affinity extramitochondrial heme-protein associated with K⁺ channels is responsible

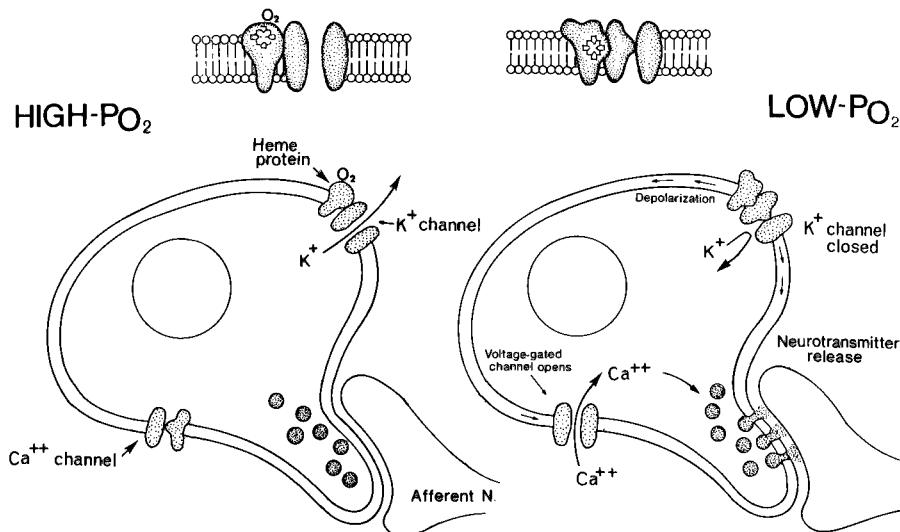


Figure 11 Model of the transduction mechanism of carotid body type I cells with a heme-protein O_2 -sensor regulating the open probability of K^+ channels and therefore depolarization of the membrane with hypoxia that opens voltage-gated Ca^{++} channels. The Ca^{++} influx stimulates neurotransmitter release to the afferent neuron. Redrawn from 25.

for responses to higher PaO_2 s and the reduced mitochondrial ATP production (via cytochrome a_3) becomes important in transducing the lower PaO_2 signals. A 'proactive' regulatory system (as Hochachka and Matheson²⁸ would call it) that would respond to declining PaO_2 well before it has any significant impact on mitochondrial respiration seems a reasonable control system and would necessarily rely upon an extramitochondrial cytochrome of much lower oxygen affinity than those within the mitochondria and of moderately higher affinity than that of hemoglobin. Nonetheless the important point for the present discussion is that all transduction mechanisms currently under consideration involve a hemoglobin-like pigment (i.e. extramitochondrial b type cytochrome) acting as an O_2 -sensor. This intracellular or plasma membrane integral heme-protein represents the potential link between hemoglobin affinity and hypoxic sensitivity if a genetic connection can be made between them.

The possible genetic connections would seem to be rather limited. One scenario is that genes for hemoglobin and the O_2 -sensor are quite distinct and the apparent 'matching' of affinities merely reflects the fact that they are subject to the same selection pressures. The many interspecific examples of matching of hemoglobin affinity and apparent O_2 -sensor affinity as reflected in the hypoxic ventilatory response could simply reflect this kind of process. However another possibility is that the genes for a family of heme-proteins including hemoglobin and the O_2 sensors do have some commonality. The sparse intraspecific data indicated on the one hand that it was possible to alter the affinity of hemoglobin without apparently altering the affinity of the carotid body O_2 sensor in the Human genetic mutation creating the Andrews-Minneapolis type of hemoglobin. On the other hand, some other genetic change that led to distinct hemoglobins in sheep also perhaps led to distinct O_2 -sensor heme-proteins in the carotid bodies as reflected in the associated change in hypoxic sensitivity, suggesting some connection between the genes for these two proteins. The intraspecific

data on the parallel effects of temperature upon hemoglobin affinity and hypoxic ventilatory response suggest there may be not only parallel oxygen affinities in the hemoglobin molecule and the oxygen-sensor molecule but parallel effects of temperature upon those affinities.

We need to know a great deal more about the nature of the heme-protein acting as the oxygen sensor in carotid type I cells and about the genetics of that molecule and of hemoglobin to know whether this speculation is remotely reasonable. When these O_2 -sensor heme-proteins can be isolated it will be interesting to compare their affinities and temperature coefficients to those of the hemoglobin of that species and to make inter-specific comparisons as well.

Blunting of HVT

One final question arises in terms of the control of breathing. That is, although this potential link between the hemoglobin affinity and the carotid O_2 -sensor affinity helps us understand variations in hypoxic sensitivity between species or individuals, what could it possibly have to do with the phenomenon of blunting of the hypoxic ventilatory response within an individual after chronic exposure to hypoxia? This phenomenon has been described in a number of species, and has been attributed to a variety of both central and peripheral controls (see Ref. 49 for review). The work of Tatsumi *et al*⁴⁴ demonstrates a reduced carotid chemoreceptor responsiveness to hypoxia as a likely cause of the attenuated ventilatory response to hypoxia (in this case in cats chronically exposed to high altitude). But what could induce a shift in the hypoxic sensitivity of the transduction mechanism? An individual is presumably stuck with the intrinsic oxygen affinity of its O_2 -sensor heme-protein in its carotid bodies just as it is stuck with the intrinsic oxygen affinity of its hemoglobin. The only way to change it would be through some sort of allosteric modulators and when we learn more about these molecules such a mechanism could be pursued. But another possibility in the case of the O_2 -sensors involved in the transduction process is essentially a 'down-regulation' in response to hypoxia - i.e. to reduce the number of PO_2 -dependent K^+ channels (while increasing the relative numbers of other types of K^+ channels) and hence their impact on membrane potential. A very recent study⁵¹ of O_2 sensitive K^+ currents in carotid bodies from normoxic and chronically hypoxic rats suggests that in the chronically hypoxic rat the reduced ventilatory sensitivity to hypoxia is associated with a reduced density of O_2 -sensitive K^+ channels and that an intracellular O_2 -sensor system mediates the expression of O_2 sensitive K^+ channels just as an intracellular O_2 -sensor system mediates the transcription of the erythropoietin gene.

Conclusions

A comparative analysis reveals a pattern of correlation between the hypoxic ventilatory threshold and the PaO_2 at which hemoglobin begins to desaturate. This represents yet another example of optimization in animal design, insuring that ventilation is not prematurely stimulated by PaO_2 levels that represent little or no compromise in oxygen content but is stimulated to elevate PaO_2 when oxygen content does begin to decline. The fundamental question in the evolution of physiological function is how multiple functions within a system can come to be so well 'matched'. The respiratory system has presented many such questions that Weibel and Taylor⁴⁸ have pursued for the last decade in their 'symmorphosis' model. This aspect of that system, namely the potential matching between hemoglobin affinity and the oxygen affinity of an O_2 -

sensor heme involved in the control of the hypoxic ventilatory response and the erythropoietic response, presents a potentially rewarding avenue for investigation. A great deal is already known about the structure and genetics of hemoglobin. If the heme-protein acting as the O_2 sensor in carotid bodies could be isolated and both proteins studied biochemically, genetically and functionally in a comparative and phylogenetically appropriate way it would offer the potential for great progress in unravelling the evolutionary mechanisms for 'matching' function.

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CHAPTER 8

THE HALDANE-BARCROFT DEBATE

Charles S. Houston, Chairman

The meeting will be in order. This is the thirteen hundred and twentieth meeting of this Society since it was founded.

The Society has the honor of welcoming Professor Joseph Barcroft and Professor John Haldane, distinguished members who will address us this evening.

Fellows will recall that on December 15th, 1922, three years ago, Professor Barcroft and Professor Haldane presented their positions in respect to the passage of oxygen from the lungs into the capillaries of the lungs. Since then several papers on this subject have appeared but there is some disagreement. One school believes that oxygen passes by simple diffusion, the other that oxygen is secreted "uphill" as it were from lung to blood.

The argument of the debate this evening is

RESOLVED THAT THE LUNGS SECRETE OXYGEN

Professor Haldane represented by Dr. West will take the affirmative, while Professor Barcroft, represented by Dr. Milledge will take the negative. The debate is governed by Oxford rules.

After the rebuttals, and the Judges' decision, the Chair will entertain comments and discussion from the floor.

The Proposition: That the Lungs Secrete Oxygen

For the Affirmative: John Haldane and John West

For the Negative: Joseph Barcroft and James Milledge

The Oxford Rules

1. The Affirmative speaks first, followed by the Negative. Then the Affirmative gives its rebuttal, followed by the Negative's rebuttal. Each side will have twenty minutes for its case, and five minutes for rebuttal.
2. The affirmative may make any reasonable definition of terms in the proposition; the negative may challenge this. The Chair will rule. Once definitions are agreed they may not again be challenged or changed.
3. The affirmative must present everything required by the proposition, and may attempt to prove whatever parts it wishes.
4. Neither side may revise its position during the debate.
5. The burden of proof is on the affirmative which must give enough evidence to prove its case. All data relevant to the case may be presented.
6. No new constructive arguments or data may be offered in the rebuttals, except that either side may present new evidence in the rebuttal if it is essential for refuting a statement by the other side. If challenged, the Chair will rule.

7. During the last rebuttal, if one side believes the other has misquoted or has introduced inaccurate facts or is violating the rules, it may appeal to the Chair, and that appeal time will not be charged against that side.

8. During the constructive presentation, either side may ask the other a reasonable number of questions. If he makes clear that this is not a rhetorical question and that he really expects an answer, the other side must answer unless he can show a good reason for not doing so.

9. Restatement or quotation of the other side's argument must be accurate. Facts, presented as facts in the debate, must be accurate.

10. A panel of three judges will be appointed in advance to decide which side won. After this decision, the audience will be asked to vote on the merits of the debate, by a show of hands. The floor will then be opened for discussion for another 15-20 minutes.

For The Motion: Professor Haldane (John West)

It is certainly a special pleasure and an unexpected opportunity to present my ideas on oxygen secretion in the lung after so long. I have to say that the winters here in Oxford seem to be more severe than I remember. Nevertheless it will be a pleasure to convince my friend Barcroft of the error of his ways.

First I should make clear that the notion of oxygen secretion in the lungs was not originally my idea, although I would be happy to take credit for it. Pflüger in Bonn attributed the notion to Ludwig in Leipzig, although in point of fact he misquoted Ludwig. Incidentally it is amusing to read the acrimonious exchanges between these competing German schools. I flatter myself that we conduct our scientific discourse in a more gentlemanly fashion, as exemplified by our discussion this evening.

The notion of oxygen secretion in biological tissue originated with the French physicist Jean-Baptiste Biot in 1807. He was studying the gas in swim bladders of fish in the Mediterranean, and attempted to analyze the composition of the gas by adding a small amount of hydrogen to a sample and passing an electric spark through the mixture. He was suitably astonished when the glass eudiometer was shattered with a violent explosion. He realized that this implied that the swim bladder gas had a high concentration of oxygen, and later showed that this was over 80%. Since the oxygen partial pressure in the swim bladder gas was far higher than in the surrounding water, he concluded that oxygen was being secreted.

This work was extended by Moreau who showed that fish could regulate gas secretion into the swim bladder to control their buoyancy. Subsequently my friend Bohr in 1894 showed that the gas production could be prevented by cutting the branches of the vagus nerve that go to the swim bladder². Since the swim bladder like the lung is a diverticulum of the gut, it is not a big step to envisage oxygen secretion in lungs as discussed in the chapter devoted to this topic in my book *Respiration* written with Priestley⁹.

Some of the best early data showing oxygen secretion by lungs comes from the laboratory of Bohr who was a pupil of Ludwig. In his 1891 paper "Über die Lungenmatung" (On Pulmonary Respiration) he compared the PO_2 in the arterial blood and alveolar gas¹. The PO_2 in the blood was determined using a haemataerometer, a device whereby a small bubble of air is exposed to the blood until equilibration of the partial pressure occurs. The alveolar gas was sampled with a thin catheter at the

tracheal bifurcation during expiration. Figure 1 shows that with the animal breathing air, the arterial PO_2 was as much as 30 mmHg above the alveolar value. In addition, the alveolar PCO_2 exceeded the arterial value by over 12 mmHg in several instances. Figure 2 shows that when the animal was breathing a gas containing carbon dioxide, the alveolar PCO_2 exceeded the alveolar value by as much as 30 mmHg. These results clearly show that the lung was secreting both oxygen and carbon dioxide against a partial pressure gradient.

A year or two after these experiments had been carried out, I visited Bohr's laboratory in Copenhagen with my good friend Lorrain Smith. We were very impressed by the experimental data. Bohr referred to the secretion ability of the lung as its "specific function" (spezifische Tätigkeit), and later showed that this required the lung to consume substantial amounts of oxygen³.

I was more interested in human lungs than animal lungs, particularly since I was studying the air in mines and was concerned about the physiological consequences of atmospheres with low oxygen concentrations. The mines frequently contained carbon monoxide and I therefore had studied this gas and its combination with blood. We conceived the idea of measuring the PO_2 in arterial blood from the relative concentrations of carboxyhemoglobin and oxygenated hemoglobin. The appropriate equation is:

$$\frac{\text{PCO}}{\text{PO}_2} \cdot M = \frac{[\text{HbCO}]}{[\text{HbO}_2]}$$

and the constant M could be measured *in vitro*. This approach obviated the need for measurements using an aerotonometer which involved many potential errors.

Our first study was published in 1896 entitled "The Oxygen Tension of Arterial Blood." In this we showed that normal arterial blood has a PO_2 of about 200 mmHg during air breathing, that is about twice the alveolar value⁶. Because of the great interest of this result, we then carried out a large series of experiments on various animals including mice, birds and dogs. The results were published in 1897 in a paper entitled "The Absorption of Oxygen by the Lungs" where we showed that arterial PO_2 always exceeded the alveolar value⁸. Thus, as we stated at the end of the paper, "the absorption of oxygen by the lungs . . . cannot be explained by diffusion alone." We further showed that reducing the inspired oxygen concentration increased the relative excess of arterial over alveolar PO_2 . We concluded therefore that the "want of oxygen acts as a stimulus to absorption of oxygen."

Regrettably, we later realized four possible sources of error in our method using carbon monoxide. First, we had used an inaccurate shape for the oxygen dissociation curve. Next, we had assumed that the constant M was the same for all species of hemoglobin, but later we showed that this was not the case. We also realized that bright light can cause dissociation of carboxyhemoglobin. Finally, there was a question of whether we had allowed sufficient time for equilibration between the carbon monoxide in the alveolar gas and the blood.

My friend Douglas and I therefore carried out a new series of experiments which we published in 1912 "The Causes of Absorption of Oxygen by the Lungs." We used the carbon monoxide techniques as before, but this time gave great attention to the potential sources of error listed above, and particularly the time required for equilibration between alveolar gas and arterial blood⁵. We used the technique of initially

loading the blood with a high concentration of carbon monoxide, and then allowing a period of equilibration during unloading when the subject was connected to a closed circuit. Equilibration periods of between 20 minutes and 1 hour were used.

The results of these experiments carried out on ourselves are shown in Figure 3. Note that at rest, the alveolar and arterial PO_2 values were essentially the same. This meant that there was no oxygen secretion at rest. However we were able to show that under certain conditions the arterial PO_2 exceeded the alveolar value. This evidence for oxygen secretion was seen in 3 situations: when we inspired a low oxygen mixture, during carbon monoxide poisoning, and during muscular work. We therefore concluded that although the lung does not require oxygen secretion under resting conditions, when an abnormal stress is placed on the organ, oxygen secretion is called into action.

The question of adequate equilibration time for carbon monoxide between alveolar gas and arterial blood has been debated at some length, and I would not be surprised if my friend Barcroft raises this old chestnut again. Let me just say that our mutual friend, Hartridge, published a series of experiments in 1912 ("Experiments on the Oxygen Secretion in the Lung of Man by the Carbon Monoxide Method") using an identical equilibration technique¹¹. He was not able to find any evidence of oxygen secretion which strongly suggests that problems with equilibration were not responsible for our results. Presumably Hartridge's different conclusions can be explained by his use of a spectroscopic method for measuring carbon monoxide¹² and its associated errors.

Of course these experiments in which carbon monoxide was inhaled in substantial concentrations involved some risk. However I would like publicly to deny the implication in my wife's autobiography that I was responsible for the death of the Italian physiologist, Professor Angelo Mosso because of a displaced decimal point in a publication in the *Journal of Physiology* which resulted in his breathing a lethal mixture¹⁰. As my son Jack remarked about his mother's biography, the mean was about right but the standard deviation was excessive.

Our most elaborate investigation of oxygen secretion was that carried out during the Pike's Peak Expedition of 1911. The investigators were Douglas and myself assisted by two Americans, Henderson and Schneider. We used the same carbon monoxide equilibration technique such that about 20% of the hemoglobin in the arterial blood was bound to carbon monoxide. Every precaution was taken to avoid errors, and the results were quite unmistakable⁴. As soon as acclimatization to the low barometric pressure was established, the arterial PO_2 became considerably higher than that of alveolar gas. Figure 4 shows the results. Under resting conditions on acclimatized persons on Pike's Peak, the arterial PO_2 was about 70% above the alveolar value. When air enriched with oxygen was breathed, the difference between the arterial and alveolar PO_2 values fell to 8 or 10%. In one subject investigated immediately on arrival at the summit, the arterial PO_2 was only about 15% above the alveolar value, whereas three days later after acclimatization, the excess was 100%.

I cannot leave our Pike's Peak Expedition without referring briefly to criticism, no doubt well intentioned, of our relationships with Miss Mabel FitzGerald who accompanied Douglas and myself from Oxford. Naturally we could not allow the unchaperoned Miss FitzGerald to share our accommodations in the small hotel on the summit. It is very much to her credit that she spent the time visiting mining camps in

Colorado accompanied only by a mule, and that she carried out some really quite important physiological measurements on the composition of alveolar gas and blood hemoglobin concentration of miners at a range of altitudes.

A final series of experiments was carried out with Kellas and Kennaway and published in 1919 ("Experiments on Acclimatization to Reduced Atmospheric Pressure"). These studies were carried out in a low pressure chamber in London where we were exposed for 6 to 8 hours to pressures of 500, 430 and 300 mmHg on three successive days⁷. These experiments showed clear evidence of acclimatization in spite of no lasting changes in alveolar PCO₂, blood reaction (pH), or hemoglobin concentration. It seems unmistakable that the acclimatization was caused by oxygen secretion by the lungs.

For those of us with a broad interest in biology, and respect for the special properties of living organisms, the fact that the lungs can actively secrete oxygen comes as no surprise. The mechanistic theory of life popularized by such masters as Huxley in the last century is now outworn and must soon take its place as a passing phase in the development of biology. The time has come for a far more clear realization of what life implies. The bondage of biology to the physical sciences has lasted more than half a century but the time has come to realize that organic regulation and maintenance represent something very real.

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TABLE 1
Experiments with inspiration of carbon dioxide-free atmospheric air

Experiment No.	Oxygen pressure				Carbon dioxide pressure				Per kilogram and hour			
	in bifurcation		in arterial blood	difference	in bifurcation		in arterial blood	difference	CO ₂ expelled	O ₂ uptake	Respiratory quotient	Remarks
	in gas	in bifurcation			gas	bifurcation	blood					
I.	127.4	143.9	+16.5	16.6	10.1	-	6.5	592	588	1.01	A. L.	
II.	132.1	142.1	+10	9.9	0	-	9.9	159	281	0.57	A. P.	
III.				24.2	10.9	-	13.3	555	664	0.84	A. L.	
IV.	131.4	105.4	-26.0	14.3	16.7	+	2.4	545	634	0.86	A. L. } A. P. }	
Va.	118.3			5.8	19.8	+14						
Vb.	116.3			9.7	20.9	+11.2		203	-	-		
VI.	95.4	101.2	+ 5.8	34.6	17.4	-17.2		454	848	0.54	A. L.	
VII.	114.1	115.9	+ 1.8	25.9	31.7	+	5.8	363	451	0.81	V. L.	
VIII.				12.0	20.5	+	8.5	417	545	0.77	V. P.	
IX.	116.8	117.9	+ 1.1	22.8	38.0	+16.8		524	823	0.63	A. P.-M.	
Xa.	103.0	141.0	+38	23.2	11.2	-12		528	881	0.60	A. L. } A. L. }	
Xb.	109.8	121.7	+11.9	14.8	27.6	+12.8		504	658	0.77	A. L.	
XI.	112.2	121.7	+ 9.5	28.4	27.7	-	0.7					

Figure 1 Table 1 from Bohr¹ showing evidence for secretion of both oxygen and carbon dioxide by lungs. The third and sixth columns show the differences between arterial blood and alveolar gas (sampled at the tracheal bifurcation). Bold numbers show where the PO₂ was higher in arterial blood than alveolar gas, and where the PCO₂ was higher in alveolar gas than arterial blood.

TABLE 2
Experiments with inspiration of gas containing carbon dioxide

Experiment No.	Oxygen pressure			Carbon dioxide pressure			Per kilogram and hour			Inspired air		
	in bifurcation gas	in arterial blood	difference	in bifurcation gas	in arterial blood	difference	CO ₂ expelled	O ₂ uptake	Respiratory quotient	CO ₂ , %	O ₂ , %	Remarks
XII.	116.1	106.1	-10	40.6	29.7	-10.9	424	817	0.52	4.9	18.8	V. L.
XIII.	130.4	143.6	+13.2	28.5	0.9	-27.6	470	423	1.11	3.2	20.0	A. L.
XIV.	127.1	127.6	+ 0.5	28.8	19.9	- 8.9	604	560	1.08	2.0	20.2	A. L.
XVa.	120.5	122.3	+ 1.8	69.8	37.4	-32.4	280	544	0.52	8.9	18.9	A. P.
XVb.	117.9	118.4	+ 0.5	72.5	57.8	-14.7	{			{		
XVIa.	-	-	-	32.2	34.9	+ 2.7	{			{		
XVIb.	-	-	-	34.4	36.3	+ 1.9	{			{		

Figure 2 Table 2 from Bohr¹. Same format as in Figure 1 but here the animals breathed a gas mixture containing carbon dioxide. It can be seen that this increased the alveolar-arterial PCO₂ difference.

TABLE 3. Experiments on arterial oxygen pressure of men.

Date	Subject	Inspired air. Gases %				Just before a meal				Into saturator at finish				Alveolar air gases %				% saturation of haemoglobin with Cl			
		O ₂	CO ₂	O ₂	CO ₂	O ₂	CO ₂	O ₂	CO ₂	O ₂	CO ₂	O ₂	CO ₂	O ₂	CO ₂	O ₂	CO ₂	O ₂	CO ₂		
14/12/10	C.G.D.	19.54	0.05	19.75	0.00	—	—	20.65	0.08	[13.6]	[5.6]	23.1	17.2	14.2	[104.4]						
14/12/10	"	22.50	0.04	—	—	—	—	22.07	0.15	15.38	5.63	23.0	16.0	14.1	91.6						
17/12/10	J.S.H.	19.57	0.02	19.72	0.02	—	—	19.75	0.06	12.46	5.51	23.5	16.6	12.8	102.7						
20/12/10	"	21.70	0.07	21.47	0.07	—	—	21.34	0.16	14.13	5.51	23.2	15.8	13.2	93.4						
21/12/10	"	25.85	—	25.38	—	—	—	22.39	0.11	14.62	5.63	22.2	17.1	16.2	110.8						
23/5/11	C.G.D.	21.78	0.02	21.13	0.10	22.64	0.13	23.19	0.20	[15.7]	[5.6]	23.85	16.35	14.45	[92.0]						
23/5/11	"	19.79	0.29	19.80	0.33	20.22	0.32	20.17	0.35	14.06	5.64	23.7	17.6	13.9	98.9						
23/12/10	C.G.D.	28.00	—	28.58	0.03	—	—	27.88	0.11	21.65	5.47	23.2	19.0	21.7	100.2						
24/12/10	J.S.H.	32.73	0.20	31.86	0.13	31.20	0.16	30.19	0.16	23.46	6.01	23.5	18.5	23.1	98.5						
11/1/11	C.G.D.	12.27	0.02	12.56	0.02	—	—	11.79	0.18	5.98	4.93	21.7	16.0	6.9	115.4						
13/1/11	"	12.82	0.02	13.61	0.03	—	—	13.34	0.11	6.99	4.78	23.0	17.7	8.5	121.6						
16/1/11	"	11.47	0.04	11.27	0.04	10.71	0.12	10.26	0.18	6.40	3.48	22.9	21.6	8.2	128.1						
23/1/11	J.S.H.	12.03	0.00	12.00	0.01	11.19	0.04	11.74	0.12	5.53	4.74	23.1	16.4	6.2	112.1						
10/2/11	J.S.H.	21.10	0.21	21.00	0.44	18.46	0.57	18.42	0.58 ¹	12.82	5.39	23.3	20.9	16.0	124.8						
11/2/11	"	21.19	0.24	—	—	20.56	0.84	21.43	0.80 ²	15.11	5.33	22.7	21.6	19.9	131.7						
27/2/11	C.G.D.	21.88	0.11	21.84	0.11	20.66	1.30	20.53	1.11	15.49	5.69	23.4	24.0	20.9	135.0						
28/2/11	J.S.H.	17.95	0.33	17.94	0.38	17.68	0.36	18.05	0.36	11.64	5.22	21.1	18.1	14.9	128.0						
27/4/11	C.G.D.	22.32	0.96	22.88	0.90	23.49	1.07	24.12	0.82	19.38	4.43	24.25	24.75	24.8	128.0						
27/4/11	C.G.D.	12.50	0.02	—	—	12.87	0.38	14.72	0.24 ³	10.20	3.30	23.5	23.9	15.1	147.6						

¹ CO₂ added to saturator to 7.02%. ² CO₂ added to saturator to 4.70%. ³ CO₂ added to saturator to 4.64%.

The figures in brackets are calculated values.

Figure 3 Table from Douglas and Haldane⁵ showing evidence for oxygen secretion. The last column on the right shows the arterial PO₂ as a percentage of the alveolar value. Note that there was no consistent difference when the subjects breathed air or high oxygen at rest. However during low oxygen breathing, or work, the arterial PO₂ was considerably higher than the alveolar values.

PIKE'S PEAK—ARTERIAL OXYGEN-PRESSURE

Date	Subject	Alveolar air Gases per cent.		Percentage saturation of blood with CO ₂		O ₂ -pressure of arterial blood in percentage of the existing atmosphere without allowing for aqueous vapour	O ₂ -pressure of arterial blood in mm. Hg (at 37° moist)	O ₂ -pressure of alveolar air in mm. Hg (at 37° moist)	O ₂ -pressure of arterial blood in mm. Hg (at 37° moist)
		O ₂	CO ₂	In vivo	In vitro				
July 19	C. G. D.	13.03	6.91	21.0	22.5	21.9	53.4	89.8	
" 20	"	11.96	6.82	20.5	22.7	20.9	49.0	85.6	
" 24	"	21.13	7.09	19.2	17.5	25.1	88.0	103.6	High O ₂ insp.
" 26	"	24.23	7.40	18.15	16.0	26.8	99.3	109.9	High O ₂ insp.
Aug. 2	"	15.80	6.58	17.5	20.75	24.0	64.6	98.1	Work
July 21	J. S. H.	16.20	6.28	19.35	20.4	24.9	66.8	102.4	
" 28	"	14.81	6.41	19.45	21.4	22.7	60.5	92.8	
Aug. 1	"	8.13	4.76	18.3	19.4	13.5	33.2	55.2	Low O ₂ insp.
" 13	"	29.20	7.40	19.0	17.3	31.7	120.6	131.0	High O ₂ insp.
July 29	Y.H.	13.77	6.67	18.0	16.7	18.8	56.9	77.7	
Aug. 9	"	16.56	5.86	16.3	18.4	25.3	68.4	104.4	
July 31	E. C. S.	10.61	7.61	19.2	21.6	20.3	43.4	83.0	
Aug. 8	"	12.70	7.63	18.6	20.8	23.4	52.3	96.4	
Aug. 4	J. E. F.	11.16	7.93	12.8	9.5	12.9	45.6	52.7	On arrival
" 7	"	9.86	7.62	16.75	19.7	19.75	40.7	81.4	

Figure 4 Table from Haldane and Priestley⁹ showing results from the Pike's Peak Expedition. The last two columns show the PO₂ in alveolar air and arterial blood. It can be seen that in all instances the arterial value was higher.

AGAINST THE MOTION: PROFESSOR BARCROFT (Jim Milledge)

Mr. Chairman, my Lords, Ladies and Gentlemen, as I rise to speak against this motion, so ably proposed by my friend Professor Haldane, I do so with considerable trepidation and indeed some reluctance for three reasons.

First because the hypothesis that the lungs secrete oxygen is a very attractive one. It is much more exciting than the opposing view that simple diffusion of oxygen down a pressure gradient is sufficient to account for the oxygen needs of the body. If my learned friend were to be correct what vistas of interesting study are opened up! Under what circumstances is oxygen secreted? What is the energy utilized? Can training or altitude acclimatization enhance secretion? A physiologist could be kept in business for life.

Second you have heard the evidence for the motion which is not inconsiderable from the work not only of Professor Haldane but some eminent continental physiologists as well.

And thirdly because I have the highest regard for the proposer of the motion. As I wrote in my review of his book, "Respiration"; "No one who turns the pages of this book, can but be impressed with the enormous advance which has been made in the physiology of respiration in the last thirty years, and the degree to which that advance has been due to Haldane's work and to the stimulating influence which he has wielded over the minds of others" myself, included. As many of you know I have worked with Professor Haldane as his junior colleague and owe him a great debt for his influence on my approach to physiology.

And yet as scientists we must not let this sort of consideration come between us and our search for scientific truth. I know that my learned friend is at one with me on this. Though I find myself in disagreement with Professor Haldane in this matter of oxygen secretion I wish to assure him and all of you that it in no way diminishes my very high regard for him as a man and as a physiologist.

Occam's Razor

Let us be clear as to the basis of this debate. Both sides are agreed that the principal mechanism for the passage of oxygen across the membranes of the lung, from air in the alveoli to the blood in the lung capillaries is by passive diffusion. We on this side of the house maintain that this mechanism is adequate under all circumstances, while those supporting the motion, maintain that under circumstances of oxygen shortage, ie under conditions of severe exercise or at high altitude, the lungs can also secrete oxygen. Occam's razor, obviously applies to this situation. "Entia non sunt multiplicanda praeter necessitatem" (Entities ought not to be multiplied except from necessity). For our purpose this can be rendered as, "Simple or single mechanisms are to be preferred to complex or multiple ones". Thus if diffusion is adequate we ought not to "multiply entities" by bringing in secretion as well. Thus the onus of proof is on the other side.

Before considering the evidence for and against secretion let me add, as I have written in my book "Lessons from High Altitude"; "At times I have heard persons speak as though there was some inherent absurdity in Haldane's theory and as though it were intellectually unworthy of the great man . . . Let me say I am quite out of sympathy with such statements. It seems to me to be a very good theory. . . The question is not whether it is a good theory but whether it is really supported by the facts".

EVIDENCE FOR SECRETION OF OXYGEN BY THE LUNG

You will have heard my friend Haldane display the evidence for the motion with his customary eloquence so I will be brief. Before the beginning of this new century although some physiologists on the continent of Europe claimed evidence for secretion, the apparatus used was not in any way accurate enough for the task in hand. The same criticism I'm afraid can be levelled against the work in this field of another great physiologist, Christian Bohr who claimed secretion not only of oxygen inwards but of carbon dioxide outwards. I must add that in other areas of his work Bohr made enormous contributions. And of course he stimulated his young colleague, August Krogh, to carry out his work with his wife Marie on this topic, of which more later.

So the only substantial work we have to consider is the series of studies from the Oxford school over the period 1896 to 1913 using the carbon monoxide method to obtain a value for arterial PO_2 . The earlier work of Haldane and Lorain-Smith was later withdrawn because they had used results from dilute ox blood to derive a value for the relative affinities of haemoglobin for oxygen and carbon-monoxide. We are left therefore with the work reported in their 1912 paper (Douglas and Haldane 1912) and the Pike's Peak Expedition (Douglas *et al* 1913).

From the sea level laboratory work reported in the 1912 paper Douglas and Haldane concluded that when breathing air or with added oxygen there was no oxygen secretion but when they lowered the inspired oxygen percentage or when they had their subjects exercise, secretion became evident. The arterial PO_2 was about 9 mmHg higher than the alveolar. Note that their subjects would have only been exposed to low oxygen for a few minutes or an hour at most.

Then we have the evidence from Pike's Peak. Fifteen experiments were conducted on five subjects. All results gave a higher arterial than alveolar PO_2 . For subjects acclimatized and breathing air the mean difference was 35.8 mmHg. When oxygen was breathed the difference was reduced to 10 to 21 mmHg. In one subject a difference of only 7 mmHg was found on the day of arrival at altitude but by the 3rd day this had increased to 40.7 mmHg. Clearly if taken at their face value oxygen secretion must be considered as a likely interpretation of these results.

Consequences of secretion

Before considering the evidence against oxygen secretion, I would like to make an observation on the consequences of oxygen secretion to the degree suggested by Haldane. If indeed secretion results in the arterial PO_2 being in the range 85 to 104 mmHg as reported from Pike's Peak, then the saturation would be greater than 95% and with the well known increase in haemoglobin concentration, the oxygen content of the arterial blood would have been actually higher than at sea level! Why then do acclimatized mountaineers feel breathless at altitude and have a reduced work capacity, and what is the stimulus for maintaining the increased red cell production?

EVIDENCE AGAINST OXYGEN SECRETION

Work of the Kroghs

Starting in 1906 August and Marie Krogh carried out a series of studies in animals using a greatly improved tonometric method for analyzing arterial blood. In all cases they found that the arterial PO_2 was lower than the alveolar by 2 to 3% of an atmosphere indicating that diffusion was adequate to account for oxygen transport. Marie

devised an ingenious method to obtain a value for the "diffusion constant" of the lung using carbon monoxide. They showed that the diffusion constant was great enough to provide the necessary oxygen intake and that it increased with exercise so that even with strenuous exercise diffusion alone was an adequate explanation for the observed oxygen intake. They did not examine the situation of reduced inspired PO_2 but Marie (1914) does consider the situation of a man on Pike's Peak and even on the Duc d'Abruzzi's expedition at 24,600 ft. Making reasonable assumptions in regard to minute ventilation, haemoglobin concentration, dead space etc. and her own estimates of DCO on exercise she calculates that diffusion alone could provide the assumed oxygen intake of about one litre per minute.

Work of Hamilton Hartridge

My young colleague Mr. Hartridge has refined the spectroscopic method for the estimation of carboxyhaemoglobin in the blood. Using this method he has repeated many of the experiments of Douglas and Haldane. In his paper of 1912 he makes it clear that he is careful to follow the Oxford school in their methods except in respect of the method for analyzing for carboxyhaemoglobin. In particular he is conscious of the problem of the equilibration time of alveolar air and blood. (which matter we may consider later as a possible source of error in estimating arterial PO_2). He draws the following conclusions:

"1. In these experiments the tension of oxygen in the arterial blood was not found to be greater than that in the alveolar air, although three methods of reducing oxygen in the tissues were tried.

- (a) By replacing O_2 by CO
- (b) By lowering the O_2 tension
- (c) By doing work

These results so far as they go do not confirm the view that oxygen is secreted by the lungs.

2. I have examined every part of the technique which by introducing errors could mask the evidence of secretion: but I have been unable to discover anything that would account for the difference between my results and those of Haldane and Douglas."

My own work

Before the Great War I had watched this debate as it were from the sidelines. However during the war we became interested in the fate of men gassed and who became cyanosed as a result. We found that placing them in an atmosphere enriched with oxygen, in an "oxygen tent" we could relieve their cyanosis. After the war it occurred to me that I could achieve the effect of high altitude by the same sort of device, a low oxygen tent if you will. Hence I had constructed a glass box in my Cambridge laboratory in which one subject could live for a few days and even exercise. By this time we had the advantage of the improved tonometric method for estimating PO_2 in blood although to obtain arterial blood samples necessitated the tying in of an arterial cannula and the sacrifice of the radial artery at the end of the experiment. Using these methods in 1920 I stayed in the glass box for six days during which the oxygen percentage of the air was gradually reduced until by the last day it reached 10.8%. Each day I made observations on myself, exercised on the stationary bicycle and took samples of expired air and alveolar air. On the sixth day as well as these observations my radial artery was cannulated and arterial blood samples were col-

lected at rest and at the end of exercise. My medical students, fearful for my safety, made a rota and kept watch over me every minute of the time, for which I am very grateful. The precious arterial samples were transported rapidly to the tonometer apparatus and analyzed by my junior colleagues. The results were, in short, that the arterial PO_2 was less than the alveolar PO_2 even under conditions of exercise while breathing 10.8% oxygen.

My learned friend has suggested that six days might not have been enough for me to develop the secretory capacity of my lungs. I would respectfully point him to his own work (Douglas *et al* 1912) when his subjects breathed low oxygen mixtures for only a few minutes and were reported as displaying secretion. If he suggests that I might be an abnormally poor acclimatizer I can only say that on a number of altitude expeditions in the Alps and Tenerife I acclimatized at least as well if not better than most. However I do concede that the altitude was simulated and not real and that a study of only one subject is less satisfactory than one with a number of subjects. Therefore with my American and British colleagues I organized an expedition to the Andes the next year.

Cerro de Pasco Expedition

We were a party of eight scientists, and were very well supported by the Railway Company who put a boxcar at our disposal. In this we built our laboratory and living quarters. We were able in this to carry out sea level observations in Lima and then to be transported with all our kit to an altitude of 12,300 ft at Cerro de Pasco in a few hours. We carried out an ambitious series of studies of which the matter of alveolar-arterial PO_2 difference was but one. The technique of arterial puncture by needle had now been perfected so that we could carry out numerous arterial oxygen analyses. With my young friend Nagahashi we had improved the method for analyzing the blood. The method was similar to that used by August Krogh. A small bubble of alveolar air was introduced into the syringe of blood, tonometered and then analyzed for oxygen and carbon dioxide. The results showed that the arterial PO_2 was not higher than the alveolar, indeed on average it was lower, though the difference was within the error of the method (about 4 mmHg). This was found even on exercise and after weeks of altitude acclimatization.

Conclusion

I trust that the house will agree that it has now been demonstrated beyond all reasonable doubt that, dull though it may be, the available evidence is not strong enough to support the theory of oxygen secretion by the lung, even in circumstances where it would be most advantageous to the body, i.e., of reduced inspired oxygen pressure and exercise. I would ask you therefore to vote against the motion.

REBUTTAL BY PROFESSOR HALDANE (Dr. John West)

Well as I expected, my good friend Barcroft is as eloquent as ever and of course it is very difficult not to be swayed by his arguments. One can expect this urbane wit from someone from our somewhat younger sister university, Cambridge. But I fear that in a number of respects we need to review some of what he has told us.

First of all he referred to Occam's razor, the principle that the simplest explanation is the most attractive under many conditions. However I must remind him that there are many instances in the body, well understood, where secretion occurs. For example, in the stomach the glands secrete acid against an enormous concentration gradient; in fact the concentration of acid in the stomach is some million times higher than in the cells lining the stomach.

And there are plenty of other examples within the body of secretion. This is true of other glands as has been well described. The fact that oxygen diffusion is the simpler explanation really is irrelevant. The issue is what do the facts show.

Now the measurements of Hartridge of course are impressive but we have to recognize that the spectroscopic technique has its errors just as any other technique does. Although presumably Hartridge made the most accurate measurements he could, in fact he was probably in error in his measurements of the amount of carboxyhemoglobin and the partial pressure of oxygen as it was calculated.

Let me turn to the glass chamber experiment. Of course that was an extremely gallant experiment as one would expect from Barcroft, and a very elaborate experiment indeed. But as has been pointed out it yielded measurements made on one person only, and how can we be sure that Barcroft is typical of everybody else. He says he does well at altitude but who would place much emphasis on an "n" of one. So I think we have to agree that there is some question about that study.

The measurements at Cerro de Pasco have been referred to but regrettably they were done on miners, and of course we all recognize the importance of lung disease in the mines particularly in a place like Cerro de Pasco where the conditions are not ideal. And of course it makes good sense that even if the lung is secreting oxygen, if there is some associated lung disease we could expect the arterial PO₂ to be below the alveolar value. So I fear we have to discount those measurements.

I think the evidence is in favor of oxygen secretion. We've looked at it extremely carefully from the very early measurements which I made with Lorrain Smith to the very extensive series with Douglas, and finally the very careful measurements made on Pikes Peak. I don't think anybody yet has come up with a possible reason for errors in the Pikes Peak measurements. People have talked a little bit about equilibration, but in fact, as I said early on, the equilibration process was done using two techniques. One I mentioned was the absorption of carbon monoxide from a closed circuit over a period of one hour which we allowed for equilibration. (I notice incidentally that my friend Barcroft did not elect to address the issue). The other was a technique where the blood was loaded with carbon monoxide and then the equilibration took place during the unloading of oxygen. As Hartridge has pointed out, if that is going to create any error at all it would be against the oxygen secretion hypothesis. So it seems to me we have explored every avenue we can for accuracy in these measurements.

And so, although it perhaps goes against Occam's razor and though it might perhaps be simpler if the lungs operated simply by diffusion, I feel the evidence is against it. I think from what we know about the human organism and biology as a whole, that nature would not choose such a crude device as diffusion in the face of situations where oxygen is in short supply. Surely it would be far more sensible for the evolutionary process to produce something with oxygen secretion as has been done in the stomach and numerous other glands. I think that the results I have mentioned from

my friend Barcroft's work, the glass chamber work on one subject only, the measurements at Cerro de Pasco, unfortunately in the presence of lung disease . . . all these measurements are flawed.

So I suggest with due deference that in this one small area my friend Barcroft is incorrect and indeed the lungs do secrete oxygen.

REBUTTAL BY PROFESSOR BARCROFT (Dr. Jim Milledge)

Mr. Chairman, as I said before, the secretion theory is a very attractive one, and it would be very nice if there were facts to support it because we could entertain all sorts of interesting experiments to follow up on that observation. But the fact of the matter is that if oxygen secretion were happening to any significant extent, it would be adequate to fully saturate the blood at high altitude as has been explained by my colleague here for the situation, for instance, on Pikes Peak. Yet as we noted at Cerro de Pasco, apart from the miners, we ourselves and the local natives all had this bluish tinge. We were clearly cyanosed, and when we drew blood from the artery it was blue in color. When we tonometered it with air it changed to a pink color. So aside from any questions of the accuracies of analysis, it was clear that this blood was not anywhere near 95 to 98 percent saturated. I think the fact that I have not presented a large amount of data should not vitiate the fact that blood is desaturated at altitude, in people, even at rest, let alone at exercise.

What I expected my friend Haldane to say in his rebuttal was what he said at the meeting of the Royal Society of Medicine a year or so ago when he took the position which was very different from before the War—namely that it was not really the arterial PO₂ that he was measuring, but rather the end pulmonary capillary blood. He actually said on that occasion:

"The diminishing oxygen saturation of the blood from an artery as altitude increases must be out of all proportion to the diminution of the mean oxygen pressure of the blood leaving the lung alveoli. It is only this latter, that is, the blood in the pulmonary capillary, that was concerned in the question of oxygen secretion and that we measured on Pikes Peak. We already knew it was useless to measure only the percentage saturation of oxygen in blood in an artery".

In other words what he is now saying is obviously quite true that his carbon monoxide measurement is of the PO₂, strictly speaking, in the pulmonary capillary. The fact that we found lower levels in the artery could be explained presumably, by some situation in which the blood failed to go through the lungs, in other words some sort of shunt. That seemed to be the position he was taking up to a couple of years ago and of course that requires that this shunt develops on going to altitude and is not present at sea level. I find this very difficult to understand. It very greatly diminishes the elegance of what is otherwise a very elegant hypothesis; elegant, but I am afraid untrue.

Therefore I ask you to vote against the motion that the lung secretes oxygen and instead that, dull though it is, the process is all due to diffusion.

**Comment by Marsh Tenney
on behalf of the Judges**

Dr. Tom Hornbein, Dr. Susan Niermeyer, Dr. Marsh Tenney

We have heard a learned debate by two great scientists, each of whom has articulated his arguments with wit and wisdom. Their equally skillful disputation made judgment of who was the winner difficult, but, in the end, one was found to be more persuasive. In our analysis we were struck by the fact that the contest was focused on the results obtained under conditions of hypoxia (there was no disagreement on the normoxic measurements) and on the relative accuracy of the different methods employed. Haldane's methods were indirect, and there was some doubt regarding the true value of the constant "M", which Haldane recognized as erroneous in his first set of experiments, but whether the new value for the second set did not suffer the same weakness was unclear. Further doubt arose when it became apparent that the arterial-alveolar values were sometimes positive, sometimes negative, as presented in his tables.

Barcroft's methods were more direct and had the advantage of confirmed reliability based on reports from other laboratories. The criticism that Barcroft's study was of one subject only (himself) does constitute a weakness in experimental design, but the proposition put forward by Haldane that Barcroft may have been an abnormal subject was considered specious. The resort to Occam's razor as a means of deciding the correct hypothesis was not thought to be useful.

It was on these grounds that the judges have reached the unanimous decision that Barcroft had made the most telling argument and is winner of the debate. It was, however, a close call.

Parenthetically, the judges wish to comment on the high intellectual plane of this debate, but express their regret that neither contestant exposed the basic weaknesses, as claimed, of the other's methods. It would have been instructive if each scientist had repeated his own experiments, but using the other's methods.

CHAPTER 9

FETAL GROWTH AT HIGH ALTITUDE

HISTORICAL, POPULATION AND INTERGENERATIONAL PERSPECTIVES ON ADAPTATION TO HIGH ALTITUDE

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Introduction

It is a truism that evolutionary adaptation to high altitude requires that one generation successfully reproduce the next. When the Spaniards entered Peru in 1632, they encountered this challenge of adaptation to high altitude. In the words of Antonio de la Calancha,

“In Potosí all children born of Spanish parents died either at birth or within a fortnight thereafter, because the great cold and freezing air would kill them; the mothers used to leave in order to give birth in the neighboring valleys and until their child was more than a year old the mothers would exile themselves from this city...”¹²

What were the causes of this apparent increase in infant mortality? Was it simply an increase in neonatal or infant deaths? Were there more frequent fetal and/or maternal complications of pregnancy that compromised the condition of the newborn? Was it a problem confined to the Spanish newcomers? Were indigenous populations also affected, perhaps to a lesser degree? Are there analogous difficulties for reproductive success encountered today by the Han (“Chinese”) colonizing Tibet? Are such challenges encountered by lowland populations moving today to mountainous regions of North and South America? What factors ultimately permitted the Spanish to successfully reproduce at high altitudes in Peru and elsewhere?

These questions address the central issue of adaptation and the processes by which adaptation to high altitude takes place. From the perspective of evolutionary biology, adaptation refers to “characteristics of structure, function or behavior that enable the individual to live and reproduce in a given environment”¹⁴. Since adaptation occurs over generations, another way to define adaptation is the ability of one generation to

produce the next. In this perspective, the population is the entity that exists over time, consisting of a series of overlapping generations engaged in the production of the next generation.

While the population is the central unit for adaptation, it is difficult to study physiological processes enabling reproductive success on the population level. We have taken the approach that the mother and child are the minimal essential unit for reproductive success. Therefore, our studies have focused on the effects of high altitude on the relationship between mother and child during the period of pregnancy and neonatal life.

Birth weight and gestational age

A consistent fall in infant birth weight with increasing altitude has been documented for nearly forty years^{10,11,19,21}. Birth weight is reduced approximately 100 gm per 1000m altitude gain (Table 1). The reduction in birth weight at high altitude has an historical significance; the first recognition that fetal growth and length of gestation were separable influences on birth weight was at high altitude¹⁰.

Several Colorado studies have sought to determine whether fetal growth retardation or shortened gestational age is the principal cause of the reduction in birth weight observed at high altitude^{10,11,19}. While there is considerable historical variation in birth weight and gestational age in the Colorado studies, gestational age differs little at low versus high altitude at any time. The weights of babies born prematurely are similar at all altitudes in Colorado before 32 weeks. However, after wk 32, the growth curves of infants born above 9000 ft and between 7-9000 ft fall below those of the 3000-5000 ft infants (Fig. 1). Thus, the Colorado studies indicate that the principal cause of the reduction in birth weight is fetal growth retardation (Table 1).

In contrast to the Colorado data, some South American studies report shortened gestational age at high compared with low altitude (Table 1). In Peru, Gonzales⁶ reports a 1.6 wk reduction in gestational age at 4340 vs 150m. In a study of higher socioeconomic status women at 150m and 4340m, Carmen Torres and Gonzales report a higher percentage of preterm deliveries but similar gestational ages as measured by weeks from the last menstrual period and clinical assessment². Other studies in South America, for example those of Haas and co-workers⁷, show similar gestational ages in carefully-matched samples at low altitude and high altitude. Data from the Himalayan region are sparse. Gestational age was similar in Tibetan women delivering at 1200m vs 3600m²⁴ but appeared to be reduced in Ladakhi deliveries at 3600m²² (Table 1).

What accounts for the discrepancies concerning the frequency of preterm deliveries at high altitudes in the North American and in at least some of the South American studies? Several factors are likely to be involved, including sampling variation, secular trends, and ethnic variation within and between populations. Sample sizes in the South American studies, typically 100-200, are much smaller than those of the Colorado studies, approaching 200,000 in the McCullough and Unger reports^{11,19}. The Colorado data are also based on complete enumeration of the entire population whereas only 25% of Bolivian and 46% of Peruvian women give birth in hospitals¹⁶. Even among hospital deliveries, accurate assessment of gestational age is often not possible. Other difficulties concerning gestational age assessment apply to the Himalayan region. For example, it is difficult to translate gestational age from the Tibetan calendar to the Western system. Gestational age was estimated from fundal height in

Table 1. Effect of high altitude on birth weight, gestational age, and neonatal mortality.

	LOW ALTITUDE					HIGH ALTITUDE				
	altitude. m	weight, gm	gest. age, wk	% preterm	neon. mortality ¹	altitude.m	weight, gm	gest. age, wk	% preterm	neon. mortality ¹
Rocky Mountains										
Lichty, 1957	1600	3035			23.4	3100	2655*			41.6*
McCullough, 1977	<2130	3166	37.0	18.2	11.9	>2740	2962*	39.0	19.2	18.5*
Unger, 1988	<2130	3235	40.0	11.6	6.0	>2740	3058*	39.5	11.5	6.5
Jensen, 1995	<2130	3297	39.5	11.2		>2740	3056*	39.0*	14.2	
Andes										
Beall 1981	600	3410				3860	3140*			
Haas, 1980	400	3427	39.0		10.6 ²	3600	3165*	39.0		9.3 ²
Gonzales, 1993	150	3178	39.8			4340	2982*	38.2*		
Carmen Torres, 1993	150	3180	39.2	9		4340	2835*	38.6	12*	
Himalayas										
Zamudio, 1993	1200	3299	39.7	7		3600	3236	39.8	7	
Wiley, 1994						3600	2764	37.8	144	

* p < 0.05

¹neonatal mortality rate = deaths within 1st 28 da/1000 live births²infant mortality rate = deaths within 1st year/1000 live births

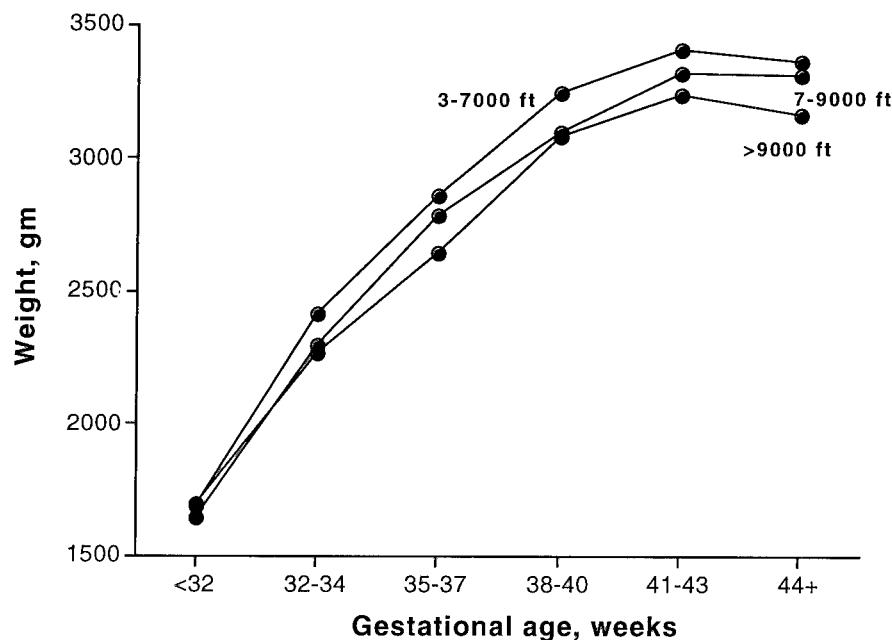


Figure 1 Fetal growth is progressively retarded after week 32 of gestation at high (> 9000 ft) compared with low (3-5000 ft) altitude in Colorado. The fetal growth response of babies born at 7-9000 ft lies between the high and low altitude curves. Data are taken from Unger et. al. (19) and represent all 179,717 live births in Colorado from 1979 through 1982.

the Ladakh study as no other estimate of gestational age was available²². Secular trends among historical periods are apparent yet some of the South American altitude samples have been obtained in different years. Ethnic and other sources of variation affecting pregnancy duration (such as the availability of prenatal care, medical interventions for preventing preterm births, and maternal nutrition) characterize the South American and Himalayan regions.

Another consideration for assessing the birth weight differences between populations is the duration of the population's high-altitude exposure. Populations have resided at high altitude for no more than 150 years in the Rocky Mountains, approximately 10,000 years in the Andes, and the longest period of time in the Himalayas. In a comparison of birth weight differences between persons from the same ethnic group who resided at low versus high altitude, Zamudio²⁴ observed that the birth weight differences were greatest in North America, intermediate in South America, and least in Tibet, suggesting that the birth weight decline at high altitude varies in relation to duration of residence. We have extended these observations within Tibet and found little change in birth weights of babies born to Tibetans across a 2700m to 4000m altitude range.

Maternal and fetal morbidity

Does the decline in birth weight signal an increase in maternal and/or fetal complications of pregnancy? If so, do such complications contribute to the reduction in birth weight observed?

We became aware of an increased frequency of preeclampsia during our studies of maternal oxygen transport at high altitude¹³. Preeclampsia remains the leading cause of maternal mortality and a significant contributor to infant mortality in the industrialized world. Preeclampsia is characterized by an elevation in blood pressure accompanied by proteinuria and/or upper extremity edema in a woman who is normotensive when nonpregnant. Hepatic, coagulation, and central nervous system abnormalities are sometimes observed as well. If untreated, preeclampsia can lead to preterm labor and delivery and, albeit rarely, maternal convulsions, intravascular coagulation, and death.

Recently, we confirmed an increased incidence of preeclampsia at high altitude in a statewide records review⁸ and in a cohort study at low versus high altitudes in Colorado¹⁵. The studies of uterine blood flow in normal pregnant and preeclamptic women at high altitude in Colorado, reviewed by Zamudio in the next chapter²⁵, suggest that the increased incidence of preeclampsia may be attributable to an abnormal blood flow adjustment to pregnancy. Identifying the contribution of alterations in hormone concentrations²⁶, vascular growth⁹, and vasoreactivity²⁰ has begun but is not completed; the complexity of vascular alterations in pregnancy is apparent in the chapter by Pearce in this volume¹⁷. To our knowledge, there has not been a well-controlled study of the incidence of preeclampsia at high versus low altitude in the Andean or Himalayan regions.

Other complications of pregnancy are more common at high altitude. Quintana¹⁸ found a three-fold increase in the occurrence of placental abruptions in 4,477 deliveries over a 15-year period in La Oroya, Peru (3750m). There was an age- and parity-associated increase such that 6.8% of women over 40 yr and 3.4% with parity greater than four had placental abruptions. The placental abruptions were associated with maternal bleeding, anemia and shock, resulted in emergency Cesarean deliveries, and accounted for 14% of the low birth weight and 12% of the preterm deliveries.

We found that more women at high than low altitude developed pregnancy complications other than hypertension in the cohort study in Colorado. At low altitude, 20% of all women and 28% of preeclamptic women developed complications other than hypertension whereas at high altitude, the corresponding figures were 30% and 66%¹⁵.

Maternal mortality in Colorado today is less than 1 death/10,000 livebirths³. While it is likely that the placental abruptions observed in La Oroya were associated with some maternal deaths, data concerning maternal mortality were not reported. Maternal mortality in Bolivia (33.2 deaths/10,000 livebirths) and Peru (30.3/10,000 livebirths) is more than twice the South American average of 14.6 deaths/10,000 livebirths (or that of any other South American country with the exception of Paraguay), but data are not available for the different altitude zones within the countries¹⁶.

The contribution of the increased incidence of preeclampsia and other complications of pregnancy to the reduction in infant birth weight at high altitude is unclear. Jensen⁸ found in Colorado that birth weights were lower in preeclamptic than normotensive women but the decline in birth weight from low to high altitude was similar in the normotensive and preeclamptic women. However, Palmer¹⁵ observed a greater fall in birth weight among normotensive women at high than low altitude who developed complications other than hypertension, suggesting that complications of pregnancy may act additively to decrease birth weight at high altitude. Clearly, further study is required to determine the contribution of preeclampsia in combination with other prenatal complications to the altitude-associated reduction in birth weight.

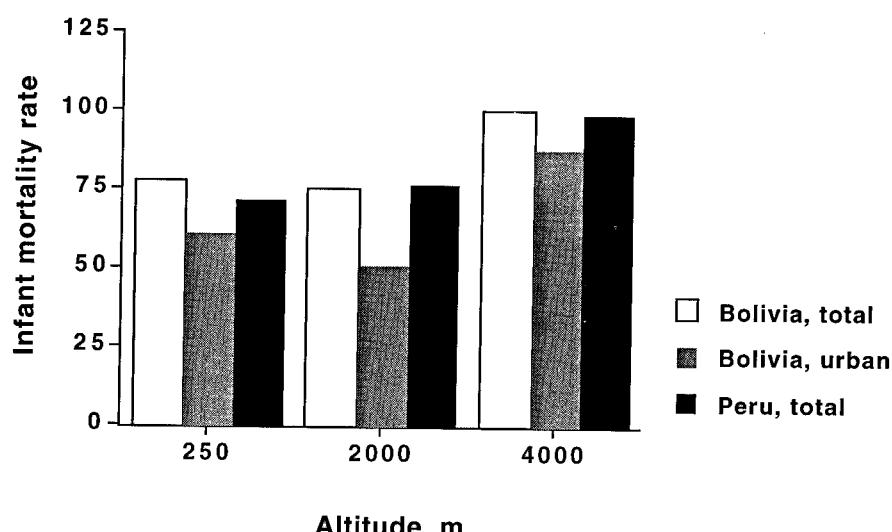


Figure 2 Infant mortality (number of deaths/1000 live births) increases with altitude in Bolivia and Peru. Data are shown for all infant deaths in Bolivia and Peru as well as the urban regions of Bolivia (16).

Infant mortality and morbidity

Does the increased occurrence of maternal or fetal complications and, in particular, the altitude-associated reduction in birth weight compromise reproductive success?

Twenty years ago, infant and neonatal mortality were above nationwide levels in Colorado and the other mountain states and rates increased with altitude within Colorado (Table 1). Today, infant mortality in Colorado is the same as nationwide and does not increase with altitude within the state^{19,21}. The cause of the mortality decline in Colorado was associated with a modest increase in birth weight, and a fall in percent preterm births¹⁹ but much of the decline remains unexplained. Being small (reduced birth weight) *per se* does not reduce infant mortality, as has been suggested¹, since low birth weight infants are at an increased mortality risk at every altitude in Colorado¹⁹ and nationwide²¹. The mortality decline occurred during the advent of specialized tertiary treatment centers and prenatal transport from remote areas. This likely contributed to the reduction in infant mortality in Colorado in general and in the high-altitude areas in particular. The highest (>9000 ft) and lowest (<5000 ft) altitude regions of the state are equally distant from the tertiary care facilities, all of which are located in the Denver metropolitan region. Yet twice as many women from the highest- compared with the lowest-altitude areas delivered in tertiary facilities¹⁹. The increased utilization of prenatal care and more frequent occurrence of pregnancy complications in the high-altitude regions may have led to more frequent referral to tertiary hospitals. Thus, today the modest decrement in birth weight with increasing altitude seen statewide in Colorado has little effect on mortality²¹; however, more pronounced reductions in birth weight among high-altitude inhabitants elsewhere are associated with a clear rise in infant mortality.

Bolivia and Peru currently have the highest infant mortality in South America and consistently have had the highest rates over the past 20 or more years¹⁶. While rates

have fallen throughout South America, the rate of fall in the Andean countries is similar to that observed elsewhere. Within both countries, there is an altitude-associated rise in all infant deaths as well as in urban infant mortality (Fig. 2). There is less of an altitude gradient for childhood mortality¹⁶, suggesting that altitude has a greater influence on factors relating to infant than childhood deaths. The infant and childhood mortality data from Bolivia and Peru is, however, of poor quality. Payment is required to register a birth or death and only about one-third of the deaths are certified by a physician¹⁶. Such problems are particularly acute in rural regions and other settings where infant mortality is likely to be highest.

In Ladakh, there is very high neonatal mortality which, in turn, is strongly related to birth weight (Fig. 3, Table 1). Respiratory disease is the principal cause of death²². There is marked seasonal variation such that the lowest birth weight and greatest mortality from respiratory disease occur in the winter. Further studies like those described by Niermeyer in this volume¹⁴ would be informative about the factors responsible for the high mortality observed.

Other neonatal complications also appear to be more common at high altitude; an altitude-associated rise in neonatal asphyxia in Peru² and other neonatal complications in Colorado have been reported^{15,19}. Cardiovascular development may also be altered in more subtle ways in keeping with the observations of Gilbert in this volume⁵.

Summary and conclusions

There are a range of maternal, fetal, neonatal and infant challenges to reproductive success at high compared with low altitude. On the maternal side, there is an increased occurrence of preeclampsia, placental abruptions, other complications of pregnancy, and preterm deliveries. The fetus is more likely to experience intrauterine growth retardation, preterm delivery, and altered cardiovascular development. Neonatal and infant well-being is adversely affected by the high mortality rates experi-

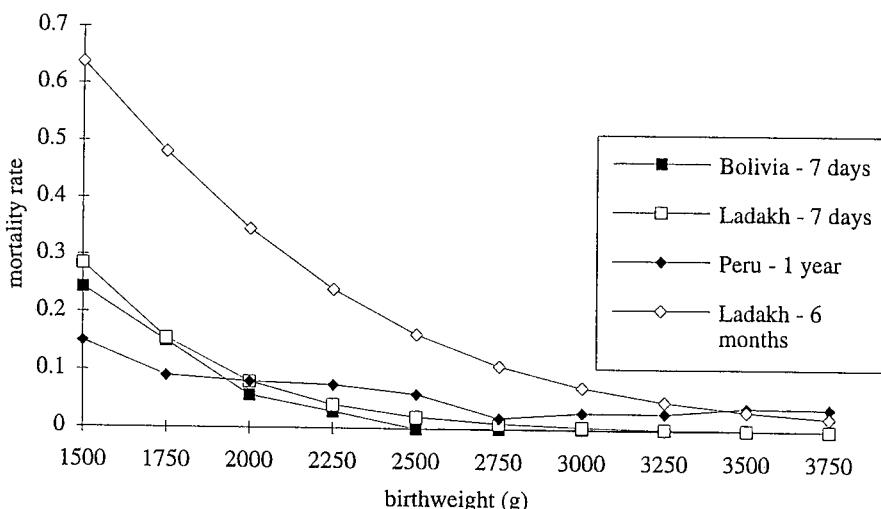


Figure 3 Neonatal or infant mortality, expressed as a probability, rises with decreasing birth weight to a greater extent in Ladakh than in high-altitude Peru or Bolivia (from 22). Copyright ©1994. Reprinted by permission of John Wiley & Sons, Inc.

enced by the majority of contemporary high-altitude residents and, in all likelihood, during the major portion of human existence at high altitudes.

The underlying causes of the challenges to reproductive success at high altitude are complex but offer insight in at least three areas; physiological, public health, and evolutionary. In the physiological area, studies of maternal, fetal and neonatal response to high altitude provide information concerning the underlying mechanisms governing the oxygen transport system. In the public health area, an opportunity exists to determine the relative importance of intrauterine growth retardation, preterm delivery, preeclampsia, and other complications for deciding infant mortality. While we recognize that a great many factors are likely involved, our ability to predict which interventions will have the most impact for specific kinds of health problems remains poor. The evolutionary importance is concerned with the inter-relatedness of maternal and fetal outcomes. As indicated above, fetal development is clearly affected by and, perhaps to a lesser extent, affects maternal characteristics. There appears to be an evolutionary hierarchy, however, insofar as the delivery of a growth-retarded and/or preterm fetus at high altitude appears to compromise survival of the infant more than the mother. Such an evolutionary hierarchy may also be operative under the circumstances of preeclampsia insofar as when untreated, preeclampsia usually leads to preterm delivery which, in turn, is the only completely effective "cure" for the disease.

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CHAPTER 10

UTERINE BLOOD FLOW AT HIGH ALTITUDE

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INTRODUCTION

Altitude-associated intrauterine growth retardation (IUGR) was first reported in North America in 1957, and was associated with increased infant mortality²⁴. A more recent statewide analysis revealed that infants from higher elevations in Colorado no longer suffer increased mortality, but their mothers use tertiary care facilities three times more often than low-altitude mothers³⁸. Because indices of reduced maternal O₂ transport are associated with lower birth weight infants at high altitude^{28,30,41} our overall hypothesis has been that reduction in fetal O₂ supply is the cause of the altitude-associated reduction in fetal growth. Fetal O₂ supply is determined by uteroplacental oxygen supply, which is in turn determined by uteroplacental blood flow and arterial O₂ content. Since arterial O₂ content is similar or greater in women pregnant at high compared with low altitude^{28,43}, a reduction in uteroplacental blood flow is the most likely source of limitation in fetal O₂ supply.

The uterine arteries supply approximately 80% of uteroplacental blood flow in primate pregnancy⁴⁰. The studies reported here use near-term uterine artery blood flow and pelvic blood flow distribution in normal pregnancy at high vs. low altitude as indices of uteroplacental blood flow⁴³. Because altitude-associated IUGR in humans occurs during the third trimester³⁸, we asked whether uterine artery blood flow was reduced in normal pregnancy near term at high compared with low altitude. Preeclampsia is a hypertensive disorder of pregnancy which occurs more frequently at high-altitude^{13,29}. Preeclampsia is often accompanied by IUGR, and has been associated with reduced uteroplacental blood flow^{20,32}. The higher incidence of preeclampsia at high altitude permits prospective study of the disease, so we also asked whether uterine blood flow was reduced in preeclamptic women at high altitude, and whether this reduction occurred prior to the onset of hypertension. Changes in uterine artery blood flow velocity and pelvic blood flow distribution measured serially throughout

pregnancy were used to assess whether preeclamptic women differed from those who remained normotensive at 3100m⁴⁴.

MATERIALS AND METHODS

Subjects consisted of 22 residents of low altitude (1600m, Denver CO) and 30 of high altitude (3100m, Leadville CO). Seven of the high altitude women developed preeclampsia. All subjects gave informed consent to procedures approved by the University of Colorado Health Sciences Center Institutional Review Board. Low altitude subjects were studied at 35.8 ± 0.3 wk of pregnancy (range=33-38 wk) and 15.1 ± 2.2 wk postpartum. At high altitude, the 23 women who remained normotensive and the 7 who developed preeclampsia were studied at wk 11.6 ± 0.4 , 23.8 ± 0.6 and 35.3 ± 0.5 of pregnancy (range=33-39 wk) and 25.3 ± 1.8 wk postpartum at St. Vincent's Hospital in Leadville. Duration of pregnancy at the time of study was back-calculated from the infant's clinically-assessed gestational age. Of the women who smoked cigarettes while pregnant only one (at 1600m) consumed more than one-half pack per day. Twelve of the high-altitude women were born and raised at ≥ 3000 m; the remainder moved to altitude as adults and had lived there for an average of 4 ± 2 years. *Preeclampsia* was defined as a resting systolic pressure ≥ 140 mmHg or a diastolic pressure ≥ 90 mmHg with $\geq 2+$ proteinuria and/or $\geq 2+$ edema of the upper extremities on two or more occasions at least 6 hr apart in a pregnant woman who was normotensive in the nonpregnant condition¹⁰.

Instrumentation and study procedures. Blood pressure was measured in duplicate using an armcuff sphygmomanometer. Hemoglobin concentration was measured with a photometer (Aktiebolaget Leo Diagnostics HemoCue, Helsingborg, Sweden) that had been previously calibrated with samples analyzed spectrophotometrically using the cyanomethemoglobin technique. Arterial O₂ saturation was measured by ear oximetry (model 47201A, Hewlett-Packard, Waltham, MA). Arterial O₂ content was calculated as (hemoglobin concentration \cdot 1.36) \cdot (arterial O₂ saturation). Unilateral uterine artery O₂ flow was calculated as arterial O₂ content \cdot volumetric blood flow. Mean blood flow velocity (*i.e.* the average speed with which blood travels through the vessel lumen in one cardiac cycle) was measured using a pulsed-wave gated Doppler ultrasound unit developed in the Cardiovascular Pulmonary Research Laboratory at the University of Colorado Health Sciences Center as previously described³⁴. Arterial diameter was obtained using an Acuson 128 Ultrasound Doppler (Mountain View, CA) with a 3.5 MHz transducer. Blood flow velocity was measured in all subjects; vessel diameter was measured and volumetric flow was calculated in a subset of normotensive subjects (n=9 at 1600m, n=5 at 3100m). Further details concerning measurement protocols, variability and reproducibility of data, and technical issues have been previously published^{34,43,44}.

Statistics. Data are reported as the mean \pm standard error of the mean (SEM). Characteristics that were not subject to change with pregnancy (*e.g.* height, education) were compared between altitudes or within the high altitude groups using chi-square or unpaired t-tests. Differences were considered significant where $p < 0.05$. A two-factor analysis of variance with orthogonal contrasts was used to examine the effects of pregnancy and altitude among normotensive subjects, and the interaction between altitude and pregnancy. The Bonferroni inequality method was applied and the P value for reporting statistical significance was reduced to 0.0125. Linear regression was used to identify relationships between variables and these results were re-

ported as significant when $p<0.05$. Hemodynamic characteristics of normotensive vs. preeclamptic women at 3100m were compared using a linear random-effects model¹⁷. The resulting response curves were compared throughout pregnancy using Scheffe's method for multiple comparisons²⁶. Differences are reported as significant where $p<0.05$.

RESULTS

Low altitude women were older, had more years of schooling and fewer were primiparous. Normotensive low and high-altitude subjects were similar in nonpregnant weight, height, weight gain with pregnancy, the number who smoked cigarettes and prenatal visits (Table 1). The infants' gestational ages were similar but birth weight was lower at 3100m than 1600m (Table 1).

Table 1. Group characteristics

	Normotensive 1600m (n=22)	Normotensive 3100m (n=23)	Preeclamptic 3100m (n=7)
Mothers			
Age (yr)	30±1	26±1*	25±3
Height (cm)	165±1	163±2	165±2
Nonpregnant weight (kg)	59±2	60±2	65±4
Weight gain with pregnancy (kg)	15±1	14±1	14±1
No. primigravid (%)	12 (50%)	4 (17%)*	6 (85%)†
No. smokers (%)	3 (12%)	5 (22%)	2 (29%)
Education (yrs)	15±1*	13±0	15±2
Prenatal care (No. visits)	13±1	11±1	14±2
Infants			
Birth weight (gm)	3415±106	3136±79*	2582±350†
Gestational age (wk)	39.7±0.6	39.7±0.4	36.8±1.6†
Pre-term (%)	4%	4%	29%†
Small-for-gestational age	0	0	43%†

* $p<.05$, normal women at 1600m vs. 3100m

† $p<.05$, normal vs. preeclamptic women at 3100m

Normotensive women at the two altitudes had similar common iliac and external iliac arterial blood flow velocities and diameters (Figs. 1 and 2). Iliac diameters did not change with pregnancy (Figs. 1 and 2). Pregnancy increased common iliac artery flow velocity and decreased external iliac artery flow velocity at both altitudes. Volumetric flow increased in the common iliac artery and decreased in the external iliac artery at 1600m. The corresponding changes in volumetric flows at high altitude were not significant (Figs. 1 and 2).

Pregnancy increased uterine artery diameter, blood flow velocity, and volumetric flow at both altitudes (Fig. 3). However, uterine artery diameter was smaller and blood flow velocity was greater at 3100m than 1600m. Higher flow velocity did not

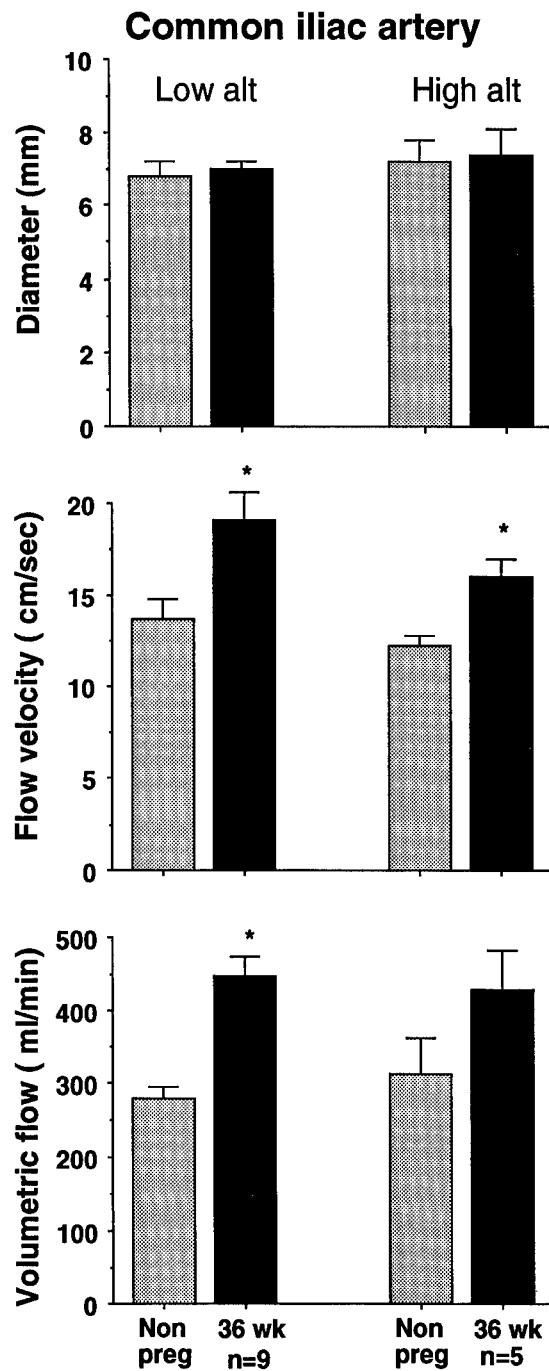


Figure 1 Common iliac artery diameters did not change with pregnancy and were similar in nonpregnant and pregnant women at 1600m vs. 3100m. Flow velocity increased with pregnancy at both altitudes. Volumetric flow increased at 1600m ($p<.005$), but not at 3100m. * $=p<0.01$ for comparison of nonpregnant vs. pregnant women.

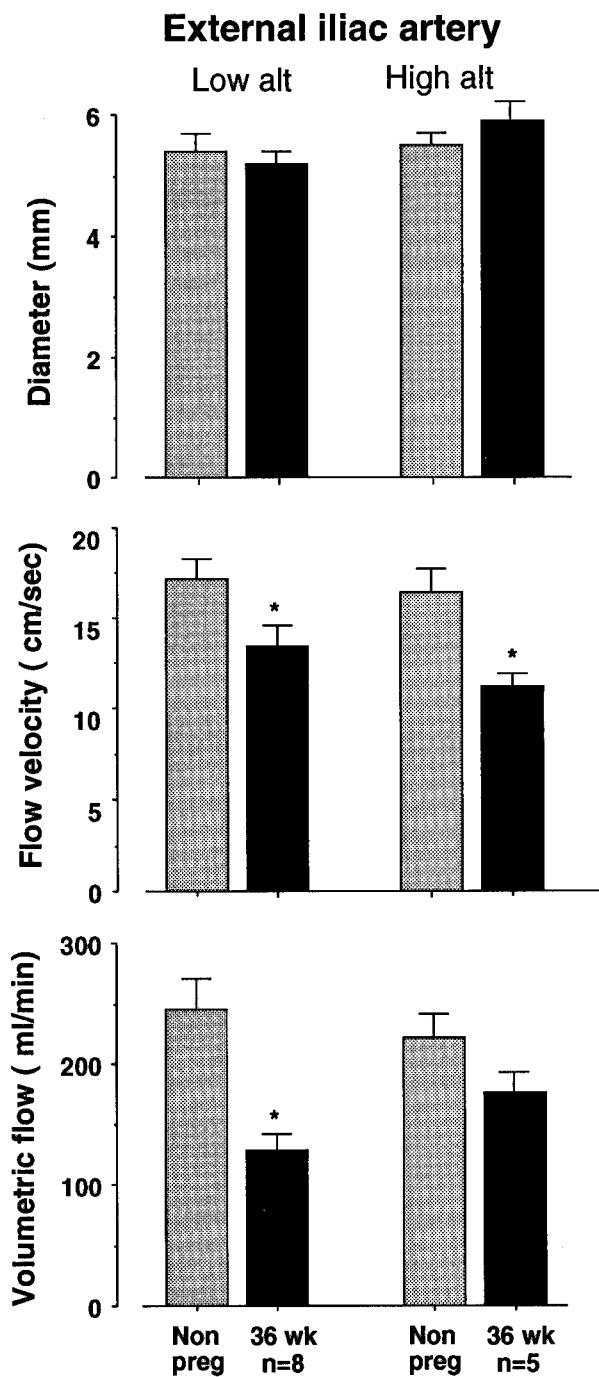


Figure 2 External iliac artery diameters did not change with pregnancy and were similar in nonpregnant and pregnant women at 1600m vs. 3100m. Flow velocity decreased with pregnancy at both altitudes. Volumetric flow decreased at 1600m ($p<.001$), but not at 3100m. * $=p<0.01$ for comparison of nonpregnant vs. pregnant women.

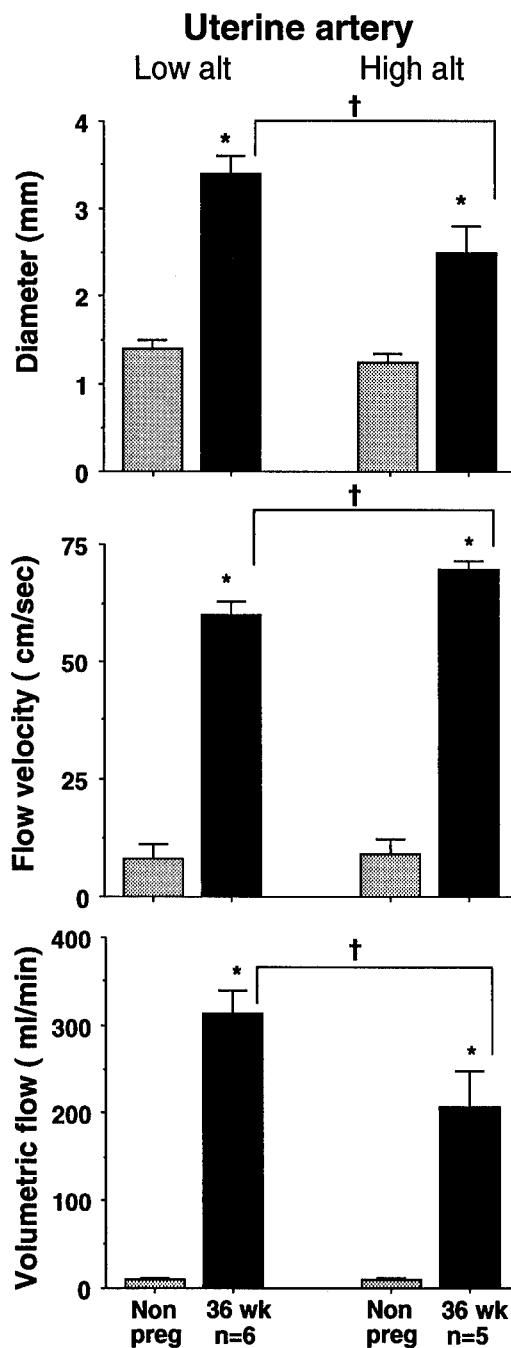


Figure 3 Uterine artery diameter, blood flow velocity and volumetric flow are greater at week 36 of pregnancy than when nonpregnant for residents of 1600m and 3100m. Pregnant women at 3100m compared with 1600m had smaller uterine artery diameter ($p<.005$), greater flow velocity ($p<.005$), and lower volumetric flow ($p<.0125$). * $=p<0.0001$ for comparison of nonpregnant vs. pregnant women. Brackets, †, $=p<.0125$ for comparisons between low vs. high altitude women.

compensate for reduced diameter, as uterine artery volumetric flow at wk 36 of pregnancy was one-third lower at 3100m (Fig. 3). Arterial O_2 content was greater in high-altitude women at wk 36 of pregnancy (17.5 ± 0.4 at 3100m vs. 16.1 ± 0.3 ml/dl at 1600m, $p < 0.01$). Still, calculated uterine artery O_2 flow was reduced by 30% at high altitude (35.4 ± 8.6 ml/min at 3100m vs. 50.5 ± 4.2 ml/min at 1600m $P = NS$). Oxygen flow per kilogram of birth weight (12.4 ± 3.6 vs. 17.2 ± 0.9 ml/min) or per kilogram of fetal weight estimated at the time of the mother's study (74.2 ± 12.1 vs. 111.8 ± 16.6 ml/min) was 28% and 34% lower at 3100m than 1600m, respectively.

Pregnancy increased the percent of common iliac volumetric flow directed to the uterine artery and decreased the percent directed to the external iliac artery at both altitudes (Fig. 4). However, less common iliac flow was received by the uterine artery in pregnant women at high altitude (Fig. 4).

Among individual subjects at the two altitudes, smaller uterine artery diameter was associated with greater uterine blood flow velocity in the pregnant ($r = -0.69$, $p < 0.05$) but not the nonpregnant condition ($r = 0.33$, $P = NS$). Smaller common iliac diameter was associated with greater blood flow velocity during pregnancy ($r = -0.76$, $p < 0.01$), but did not attain significance in the nonpregnant condition ($r = -0.56$, $P = 0.06$). The relationship was similar in the external iliac artery during pregnancy ($r = -0.54$, $P = 0.08$).

Women who developed preeclampsia at 3100m were more often primiparous when compared with those who remained normotensive, but were otherwise similar (Table 1). Birth weight was reduced among infants of preeclamptic compared with normotensive women due both to intrauterine growth retardation and reduced gestational age (Table 1).

Normotensive women at high altitude had a progressive, linear increase in common iliac artery flow velocity, uterine artery flow velocity, and the common iliac redistribution index (uterine a. flow velocity/common iliac a. flow velocity), with

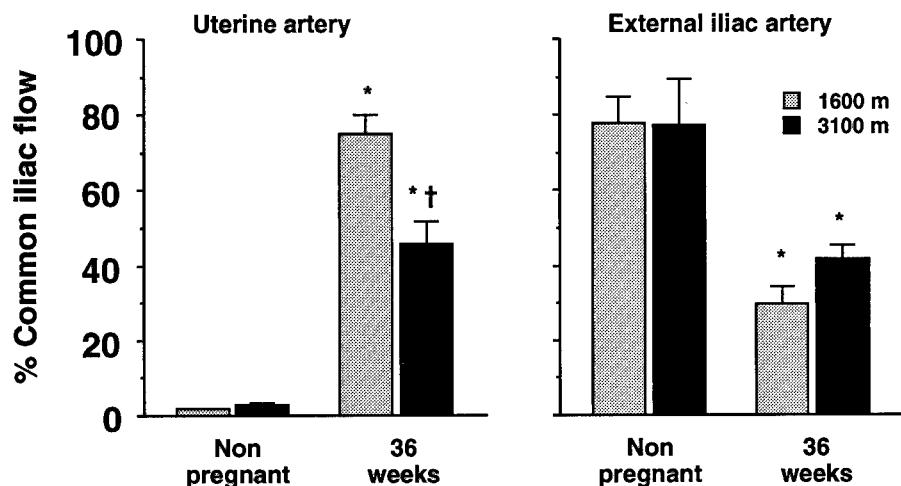


Figure 4 The percent of common iliac blood flow directed to the uterine artery increased and the percent directed towards the external iliac artery decreased with pregnancy at both altitudes. The uterine artery at 3100m compared with 1600m received less common iliac flow during pregnancy ($P < .005$). * $p < 0.005$ for comparison of nonpregnant vs. pregnant women. † $p > 0.0125$ low vs. high altitude women.

maximal values attained near term (Fig. 5). In contrast, preeclamptic women had no change in common iliac flow velocity during pregnancy and values were higher than those of normotensive women at all time points (Fig. 5A). Preeclamptic women had higher uterine artery flow velocities at weeks 12 and 24 of pregnancy, but no change thereafter, thus wk 36 values were similar to those of normotensive women (Fig. 5B). The pattern of change in the common iliac redistribution index was curvilinear among preeclamptic women; values fell between wk 24 and 36 and were thus lower than those of normotensive women at wk 36.

DISCUSSION

The effects of pregnancy on pelvic blood flow were qualitatively similar at high *vs.* low altitude. However, high-altitude residence influenced the magnitude of the changes observed. Specifically, there was less increase in uterine artery diameter and volumetric flow, a greater rise in uterine blood flow velocity, and a smaller percent of common iliac flow directed to the uterine artery at high compared with low altitude. Neither higher arterial O_2 content, nor the increase in uterine artery flow velocity in women at 3100m compensated for the reduction in uterine artery blood flow, which was 36% lower in women at high altitude. It is likely that uterine blood flow was further reduced in preeclamptic compared with normotensive women at 3100m. Preeclamptic women had no pregnancy-associated increase in common iliac artery flow velocity, no late-pregnancy rise in uterine artery flow velocity, and a fall in pelvic blood flow redistribution to favor the uterine artery during the third trimester. These departures from the pattern observed in normotensive women preceded the onset of hypertension, and support the conclusion that preeclamptic women failed to increase uterine blood flow during the third trimester.

The approximately 40% rise in common iliac artery flow velocity among normal women at both altitudes is consistent with the 40% increase in cardiac output and blood volume that occurs during normal pregnancy¹⁹. This implies that the reduction in uterine blood flow at 3100m was not due to a decrease in cardiac output. Rather, the distribution of pelvic blood flow was altered such that less “stealing” of external iliac blood flow occurred to favor the uterine artery. That stealing occurred, regardless of altitude, suggests that there is increased tone in the vasculature supplying the lower extremities during human pregnancy. Cardiac output is low and systemic vascular resistance is high in untreated preeclamptic women³⁹, which is consistent with the higher blood pressure, lower blood volume⁴¹ and lack of increase in common iliac flow velocity we observed among preeclamptic women at 3100m. A lack of increase in cardiac output and higher vascular resistance may have limited the extent to which uterine blood flow could increase among the preeclamptic subjects.

Previous studies addressing the effect of acute or chronic hypoxia on uteroplacental blood flow and O_2 delivery have been conducted nearly exclusively in sheep. In awake, chronically instrumented animals, acute hypoxia produced little or no change in uteroplacental blood flow^{7,22}. In anesthetized rabbits acute hypoxia reduced uteroplacental blood flow^{14,5}, while in anesthetized sheep both a decrease⁴ and no change in uterine blood flow have been observed¹. In sheep exposed to high altitude or lowered FIO_2 during the last few weeks of gestation, a rise in uteroplacental blood flow has been inferred from increases in uterine and umbilical PO_2 ²³. In the only study in which uterine blood flow was directly measured during chronic hypoxia, a non-significant rise in uterine blood flow was sufficient to offset a decline in maternal

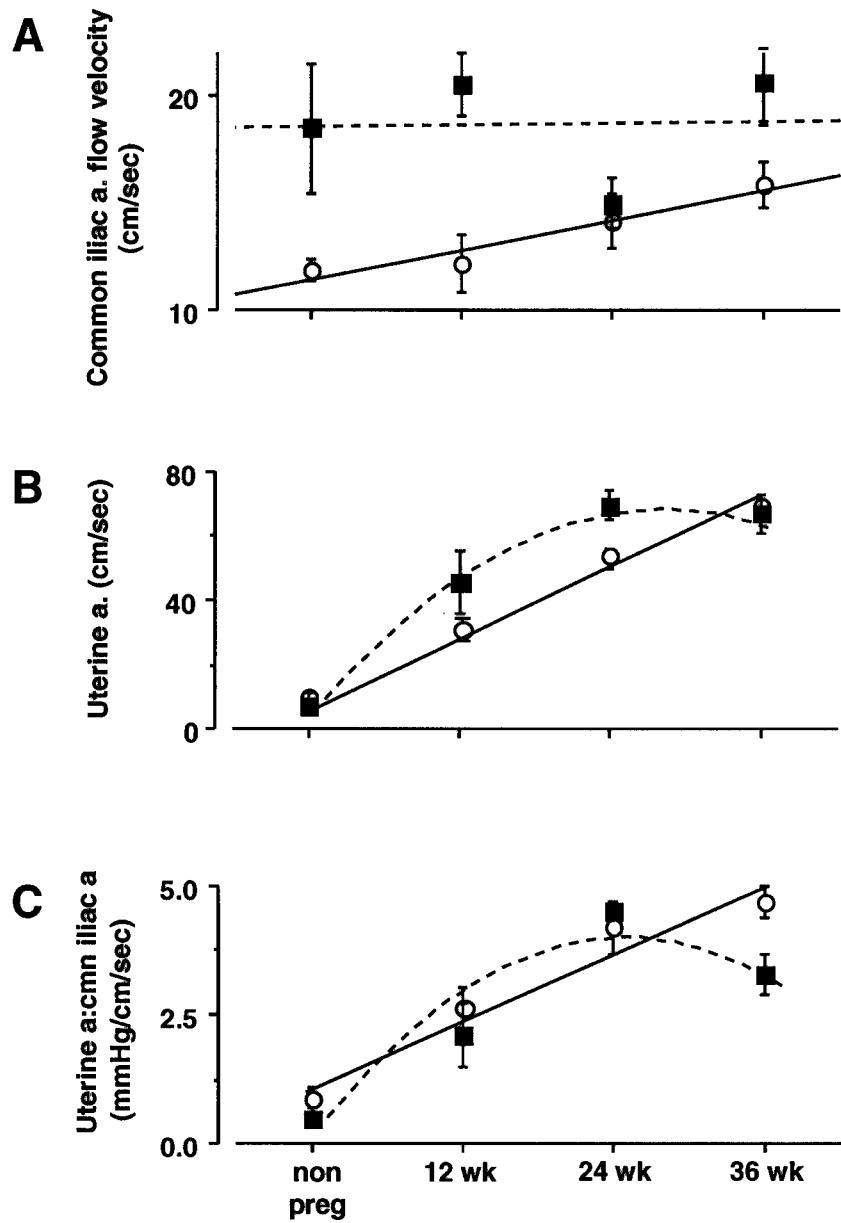


Figure 5 Common iliac a. flow velocity, uterine artery flow velocity, and the common iliac:uterine artery ratio rose progressively during pregnancy in normal women at 3100m ($p<.01$). Preeclamptic women had higher common iliac flow velocity at all times points ($p<.01$), but had no change in common iliac a. flow velocity during pregnancy. Uterine a. flow velocity was higher in preeclamptic than normotensive women at wk 12 and 24, but did not change late in pregnancy and was thus similar at wk 36. The common iliac a.:uterine a. flow velocity ratio fell between wk 24 and 36 in preeclamptic women and was thus lower at wk 36 ($p<.05$).

arterial O_2 content and maintain uteroplacental O_2 delivery at levels observed in low-altitude controls¹⁶. Only one study has been performed in sheep residing at high altitude throughout pregnancy. An increase in uteroplacental blood flow was inferred by umbilical venous O_2 tensions in the sea-level range, but the animals were surgically-stressed and there were no appropriate low-altitude controls²⁵. Moreover, the sheep were from flocks that had resided at high altitude for many generations, and did not evidence altitude-associated intrauterine growth retardation. The experimental animal literature is best summarized by noting that where maternal and/or fetal adaptations to the hypoxic stimulus favored increased O_2 transport (e.g. increased hemoglobin concentrations or PO_2), fetal growth was not retarded^{21,16,25}, whereas in their absence, birth weight was reduced¹².

A positive association between uteroplacental blood flow and infant birth weight is supported by previous human and experimental animal studies. Marked reductions in uteroplacental blood flow are required to produce significant reductions in birth weight (Fig. 6). The reduction we observed in uterine blood flow and birth weight in normotensive high altitude women was consistent with previously published data (Fig. 6). Using the regression equation relating uterine artery flow velocity to uterine

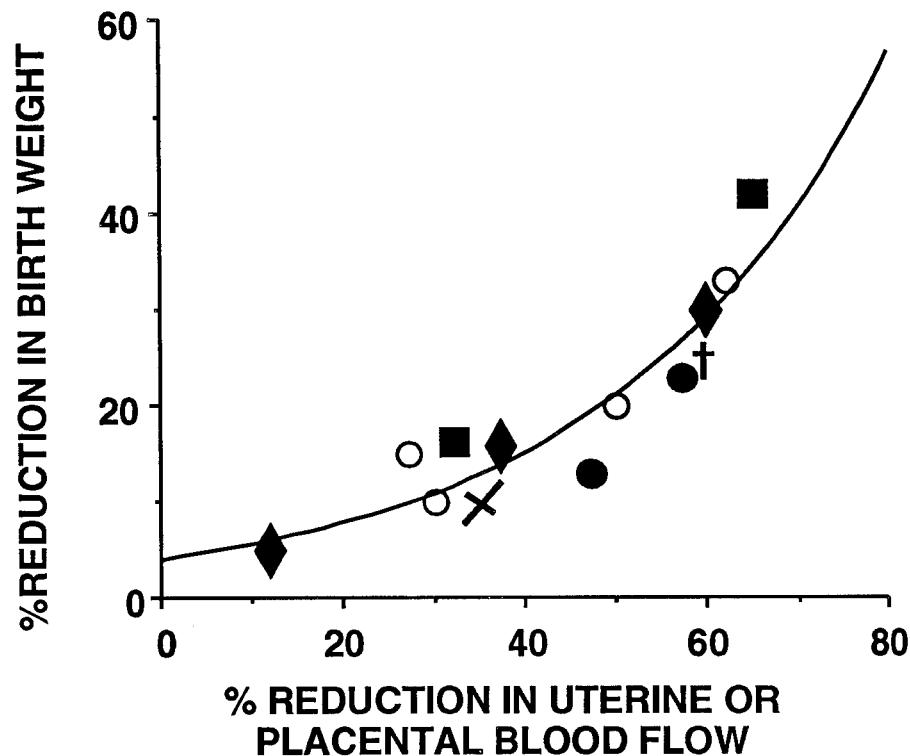


Figure 6 The percent reduction in uterine, uteroplacental or placental blood flow (X) is plotted against the reduction in birth weight (Y) reported in the literature^{2,5,6,18,20,31,32,33,36}. The best fit is exponential ($Y=3.96^*10^X(1.51-2X)$, $R^2=0.78$). The data from the present study fit the predicted curve well. (Symbols: squares=sheep; open circles=rodents; diamonds=rabbits; closed circles=humans; X=normotensive women at 3100m; †=preeclamptic women at 3100m.)

artery diameter, we estimated uterine artery blood flow was 126 ml/min in preeclamptic women, 57% less than in normotensive women at low altitude. This 57% reduction was associated with a 24% decrease in birth weight (Fig. 6).

Selective enlargement of the uterine artery is known to occur during pregnancy in humans and other species^{8,27,34}. Uterine artery blood flow in normal women at high altitude was reduced as the result of smaller diameter, not flow velocity. Because higher flow velocity was associated with reduced diameter, the elevated uterine artery flow velocities observed in the preeclamptic women at wk 12 and 24 of pregnancy imply that arterial diameters were narrower. The reduction in uterine artery diameters in high-altitude women could be due to structural differences, or to altered production of or vascular response to endogenous vasoconstrictors/vasodilators. Recent studies suggest that hyperplasia of all layers of the uterine arterial wall occurs in pregnancy and can be partially reproduced by chronic estradiol treatment¹⁵. Estradiol excretion³⁶ and circulating estradiol concentrations⁴² are reduced at high altitude as well as in preeclampsia and intrauterine growth retardation^{3,42}, suggesting that an estrogen-mediated stimulus for uterine arterial growth may be diminished under conditions of chronic hypoxia⁴². Pregnant animals housed at high altitude have increased systemic vascular resistance and isolated vessel contractile responsiveness compared with low-altitude animals^{9,11}. Thus both differences in the hormone-stimulated structural remodeling of the uterine artery and in vascular responsiveness may account for our findings.

We concluded that altitude influenced the effect of pregnancy on uterine artery diameter and redistribution of common iliac flow from the external iliac to the uterine artery, and resulted in decreased uterine blood flow. The lower birth weights associated with reduced uterine artery volumetric flow were consistent with the relationships reported in other human and experimental animal studies. Among normal women at 3100m there was progressive increase in variables which contribute to a rise in uterine blood flow. These changes were attenuated or absent in women who developed preeclampsia, resulting in no change or decline in pelvic blood flow velocities and redistribution to favor the uterine artery after wk 24 of pregnancy. Many of the physiological changes of pregnancy which may serve to increase uterine blood flow (e.g. increase in cardiac output and blood volume) attain maximal values in the mid-third trimester^{19,37}. It is possible that the rise in blood pressure which occurs during the third trimester in all pregnant women may increase perfusion pressure and thereby increase uteroplacental O₂ delivery. We speculate that hypertension may be a response to, rather than a cause of, lowered uterine blood flow and fetal O₂ delivery. It is thus possible that when maximal uterine blood flow is attained relatively early in pregnancy, increase in maternal blood pressure will be greater, leading to the organ system damage diagnostic of preeclampsia. Given this, the increased incidence of preeclampsia at high altitude may be due, in part, to a generalized altitude-associated reduction in uterine blood flow.

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CHAPTER 11

CEREBROVASCULAR DEVELOPMENT AT ALTITUDE

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Abstract

The purpose of this chapter is to briefly review the effects of altitude hypoxia on fetal cerebrovascular development. The most significant effect of chronic hypoxia on cerebral artery structure and composition is to increase protein content, and this effect is observed in both fetal and adult cerebral arteries. Hypoxia also depresses contractile responses to potassium and exogenous amines, and this effect is again observed in both fetal and adult arteries. Opposing differences between fetal and adult artery responses to hypoxia become evident upon examination of responses to adrenergic nerve stimulation. There, hypoxia enhances fetal responses but depresses those in adult arteries. Some of these age-related differences are due to enhancement of NE sensitivity in fetal arteries and depressions of NE sensitivity in adult arteries, but presynaptic effects are also involved. Presynaptically, hypoxia tends to increase innervation density and neuronal NE content, but tends to depress nerve recruitment in adult arteries and enhance it in fetal arteries. Furthermore, these presynaptic effects are similar for both the norepinephrine and the neuropeptide Y contained within adrenergic nerve terminals. Although hypoxia tends to depress neurogenic vasodilation in both fetal and adult arteries, it has opposite effects on endothelium-dependent vasodilatation in the two age groups. It enhances endothelial vasodilator capacity in adult arteries, and depresses it in fetal arteries. Superimposed on these effects are artery and age-specific changes in acetylcholine receptor density and distribution. Hypoxia has only a mild depressive effect on vascular responses to directly applied nitric oxide, and these effects are equivalent in both fetal and adult arteries. Clearly, hypoxia has multiple important effects on fetal cerebrovascular development, and also on cerebral artery function in adults.

Introduction

The purpose of this chapter is to briefly review the effects of altitude hypoxia on fetal cerebrovascular development. To that end, it will include data collected from experiments using cerebral arteries from term fetal and non-pregnant adult sheep

Additional Index Terms

A23187, acetylcholine, endothelium, neurogenic vasoconstriction, norepinephrine, neuropeptide-Y, nitric oxide, wall thickness, vascular protein, water content

maintained at either sea-level or at the White Mountain Research Station in White Mountain, California where the altitude is 3820 meters, or approximately 12,470 feet. Each summer our research group takes several dozen time-dated pregnant ewes to the station where they are maintained during their last 110 days of pregnancy, along with age-matched non-pregnant controls. Arteries harvested from these animals, and their corresponding normoxic controls are analyzed using a variety of biochemical, physiological, and pharmacological techniques to evaluate the combined effects of maturation and chronic hypoxia on artery structure and composition, contractility, and relaxation characteristics.

Effects of altitude hypoxia on artery structure and composition

One of the main artery characteristics we have routinely examined to gain insight into changes in structure is artery wall thickness. This simple measurement was achieved using projection microscopy of thin serial sections of the arteries. Results of such measurements have indicated average normoxic values in common carotid, basilar, posterior communicating, and middle cerebral arteries of 937 ± 17 , 112 ± 2 , 140 ± 5 , and $123 \pm 2 \mu$ respectively in the adult and 459 ± 15 , 85 ± 4 , 109 ± 4 , and 101 ± 2 in the fetus. Thus, as one might expect, fetal arteries were always thinner than their adult counterparts, and interestingly, this age-related difference diminished as we moved toward the microcirculation. Although chronic hypoxia tended to have opposite effects on wall thickness in fetal and adult arteries, none of these effects of chronic hypoxia were statistically significant³.

Another characteristic we routinely examined was artery protein content. For this measurement we homogenized the arteries in trichloroacetic acid, centrifuged them, and then extracted the pellet with 1 N NaOH at 37°C for one hour. Protein levels were then quantitated using the Comassie brilliant blue dye assay⁵. Given the mild nature of our extraction conditions, this assay preferentially quantitated base-soluble cellular proteins and generally excluded extracellular or matrix proteins. Protein values obtained in this manner and normalized relative to total artery dry weight are shown in Figure 1. In contrast to wall thickness, protein content increased with age, but only in the common carotid arteries. Also in contrast to wall thickness, and perhaps more importantly, chronic hypoxia significantly increased protein content in all arteries of both the fetus and the adult. Because these values were normalized relative to dry weight, the results imply that some other component of the artery wall, such as lipid, carbohydrate, or acid soluble protein, must have decreased in response to chronic hypoxia. Overall, these results demonstrated that chronic hypoxia brings about important changes in cellular protein levels regardless of age or artery type.

An additional index of artery composition we regularly examined is water content, measured as the weight change due to dehydration. Results of such measurements indicated average normoxic water contents in common carotid, basilar, posterior communicating, and middle cerebral arteries of 74.8 ± 1.3 , 78.2 ± 0.7 , 79.3 ± 0.8 , and 76.5 ± 0.7 percent wet weight, respectively in the adult and 79.7 ± 1.1 , 81.0 ± 1.4 , 78.9 ± 1.9 , 80.0 ± 0.8 percent wet weight in the fetus. Although fetal arteries generally contained more water than their adult counterparts, their water content was not significantly affected by chronic hypoxia. Similarly, water content in adult arteries was also unaffected by chronic hypoxia³. Thus despite hypoxic changes in cellular protein levels, neither wall thickness nor water content changed in either age group in response to chronic hypoxia.

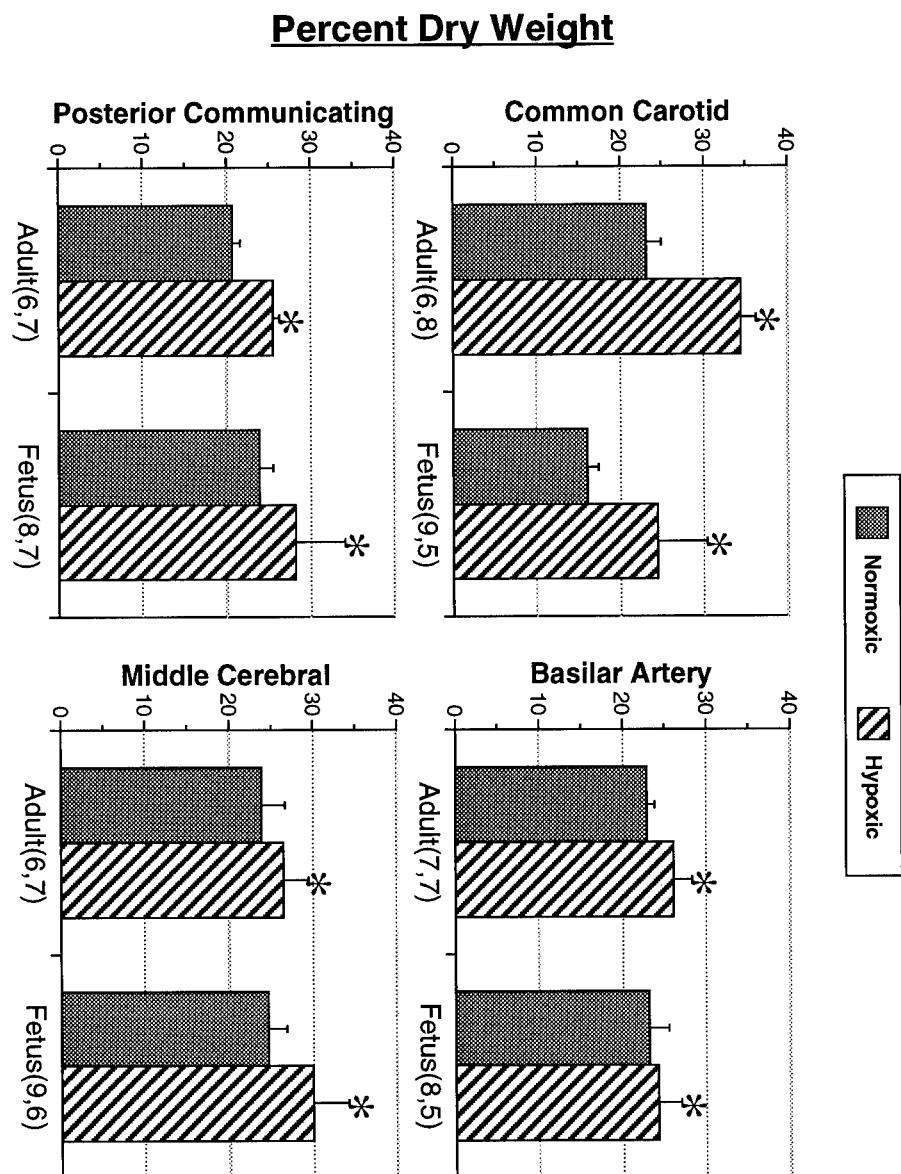


Figure 1

Figure 1 Effects of maturation and chronic hypoxia on protein content:
 Shown here are average base-soluble cellular protein levels, expressed as percent dry weight, measured in our four standard artery types from term fetal (138-143 d gestation) and non-pregnant adult sheep maintained at either sea level (normoxic) or at 3280m (hypoxic) for 110 days (the last 110 days of gestation for the fetuses). Vertical error bars indicate standard errors for the numbers of animals indicated in parentheses. Asterisks indicate statistically significant differences between fetal and adult arteries at the $P<0.05$ level (ANOVA). Note that chronic hypoxia significantly elevated protein levels in all arteries of all age groups.

Effects of altitude hypoxia on contractile responses to potassium

Because the changes in relative protein content caused by chronic hypoxia were similar in fetal and adult arteries, it was tempting to speculate that any changes in artery function produced in response to hypoxia might also be similar in fetal and adult arteries. One way to examine this idea was to compare the effects of chronic hypoxia on contractile responses in fetal and adult arteries. For such examinations, contractile responses to potassium depolarization serve as good indices of maximum contractile capacity independent of membrane receptor densities. Similarly, contractile responses to exogenous amines, such as norepinephrine and serotonin, serve as good indices of membrane receptor coupled mechanisms. And finally, responses to transmural electric field stimulation serve as more physiological indices of the functionality of these receptors and the nerves that innervate them.

When arteries from fetal or adult sheep were mounted in vitro for contractility studies and contracted with an isotonic Krebs solution containing 120 mM potassium, fetal arteries produced far less tension than their adult counterparts. Maximum tensions developed in response to potassium in common carotid, basilar, posterior communicating, and middle cerebral arteries averaged 5.1 ± 0.5 , 2.4 ± 0.9 , 1.7 ± 0.2 , and 2.2 ± 0.3 g in the normoxic fetus and 13.5 ± 1.0 , 2.5 ± 0.2 , 2.2 ± 0.2 , and 2.5 ± 0.2 g in the normoxic adult. Corresponding hypoxic values were 4.2 ± 0.5 , 1.2 ± 0.2 , 1.3 ± 0.2 , and 1.9 ± 0.2 g in the fetus and 10.1 ± 1.8 , 1.6 ± 0.3 , 1.4 ± 0.3 , and 1.9 ± 0.4 g in the adult. Although chronic hypoxia significantly increased protein content in all arteries of both age groups, it also significantly depressed maximum tension in all arteries.

To better understand if the effects of hypoxia were dependent on either wall thickness or age, we corrected for age-related differences in artery wall thickness and re-expressed the contractile responses to potassium in units of force per cross-sectional area. These values (in 10^6 dynes / cm^2) averaged 0.34 ± 0.03 , 0.43 ± 0.05 , 0.47 ± 0.03 , and 0.44 ± 0.03 in the normoxic fetus, 0.25 ± 0.02 , 0.26 ± 0.02 , 0.42 ± 0.03 , and 0.47 ± 0.05 in the hypoxic fetus, 0.32 ± 0.02 , 0.38 ± 0.03 , 0.47 ± 0.04 , and 0.39 ± 0.03 in the normoxic adult, and 0.34 ± 0.04 , 0.41 ± 0.06 , 0.49 ± 0.07 , and 0.47 ± 0.04 in the hypoxic adult. Thus, when contractile responses to potassium were compared in units of force per cross-sectional area, chronic hypoxia had no significant effect in any of the adult arteries, but significantly depressed contractility in all but the middle cerebral arteries of the fetus. Overall then, in terms of contractility, chronic hypoxia appears to have an age-dependent differential effect on responses to potassium.

Effects of altitude hypoxia on contractile responses to endogenous norepinephrine

Given that 120 mM potassium is a most unphysiological stimulus, we repeated this comparison using exogenous amines to induce tone, and obtained a similar result: chronic hypoxia inhibited contractile responses to exogenous amines only in arteries of the fetus³. Thus, we decided to further examine the effects of chronic hypoxia on contractile responses to neuronally released norepinephrine. Using previously described methods⁴, the nerves within the artery wall were directly and selectively stimulated at physiological frequencies causing release of neurotransmitters and measurable contractile effects. For all arteries, normoxic responses were significantly greater in adult than newborn arteries (Fig. 2). In sharp contrast, hypoxia enhanced all fetal responses to nerve stimulation, whereas it depressed these responses

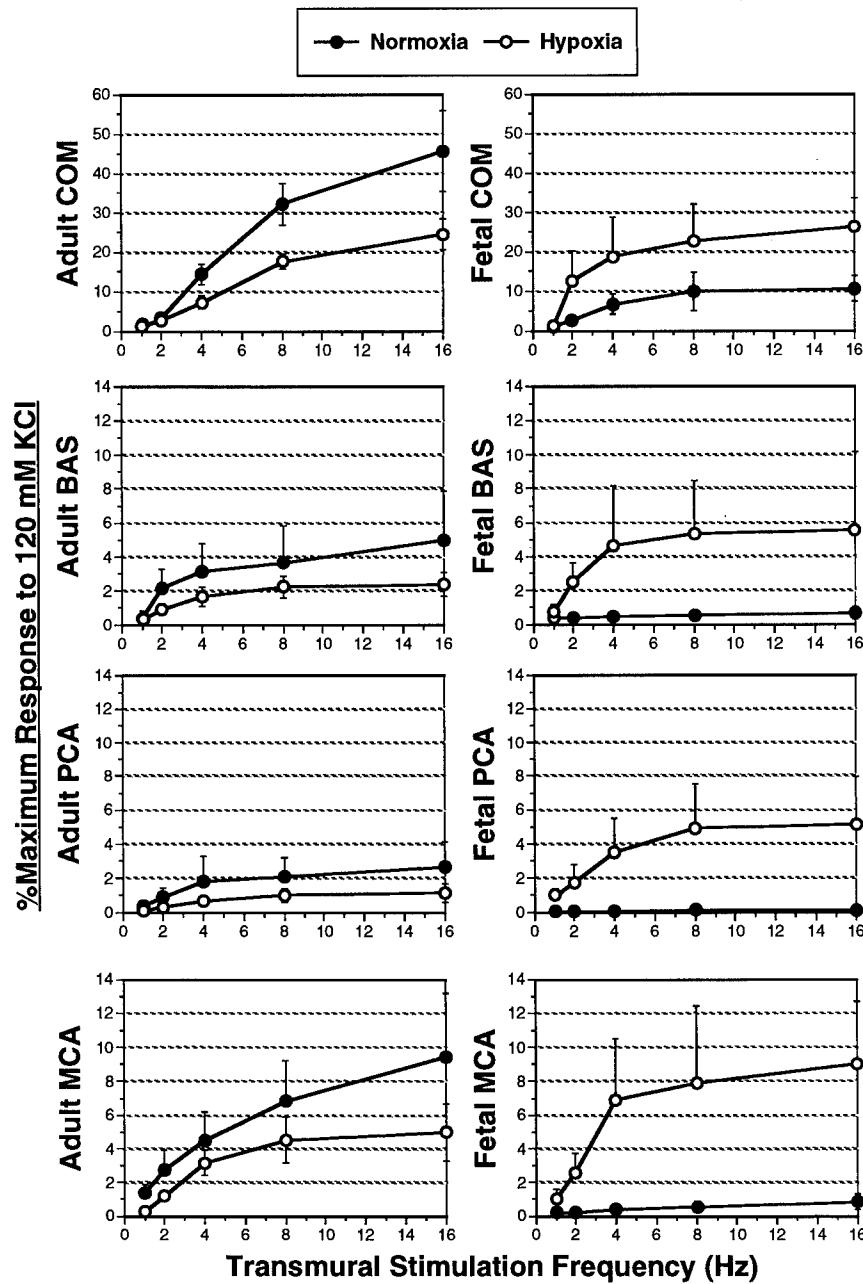


Figure 2 Effects of maturation and chronic hypoxia on stimulation-induced vasoconstriction: Shown here are the results of transmural stimulation-response experiments carried out in adult and fetal common carotid, basilar, posterior communicating, and middle cerebral arteries from animals maintained under either normoxic or hypoxic conditions. The vertical error bars indicate standard errors for a minimum of eight animals in each group, stimulus frequency is indicated on the x-axis in hertz, and the contractile responses are indicated on the vertical axes, normalized relative to the maximum response to potassium in each artery. All responses to transmural stimulation were blocked by $0.1 \mu\text{M}$ tetrodotoxin, thus indicating that they were mediated by direct neuronal activation.

in all adult arteries. Here again, then, is evidence that chronic hypoxia differentially affected fetal and adult cerebral artery contractility.

To further understand how hypoxia may modulate contractile responses to nerve stimulation, we analyzed these responses in terms of their pre-synaptic and post-synaptic components. In the simplest terms, the pre-synaptic components govern the release of neuronal neurotransmitter, and the post-synaptic determine the contractile response to that neurotransmitter. One approach to study just the post-synaptic component was to examine the dose-response characteristics for the neurotransmitter involved. In the experiments shown in Figure 2 the neurotransmitter involved was norepinephrine. Shown in Figure 3 then are the results of norepinephrine dose-response experiments conducted in each of our experimental groups. Hypoxia depressed norepinephrine sensitivity in all adult arteries, but in fetal arteries hypoxia depressed it in only the middle cerebral, had no effect on the common carotid, and enhanced sensitivity in the basilar and posterior communicating arteries. Another interesting observation was that in the intracranial arteries, maturation affected only the normoxic pD_2 values, and had little effect on hypoxic pD_2 s. But the most important point was that the opposing age-dependent effects of hypoxia on basilar and posterior communicating artery contractile responses to nerve stimulation could be explained at least partially by corresponding differential effects on norepinephrine sensitivity: in these arteries, the effects of hypoxia on pD_2 and responses to nerve stimulation were directly

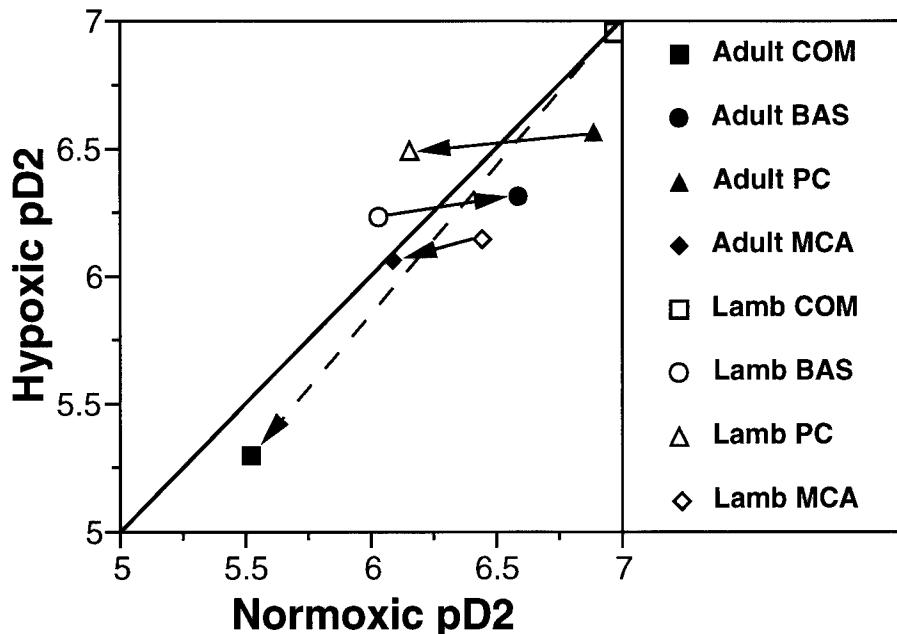


Figure 3 Effects of maturation and chronic hypoxia on norepinephrine pD_2 values:
 In this plot, corresponding normoxic and hypoxic pD_2 values, which are simply the negative logarithms of the ED50s of the dose-response curves, are plotted against each other for each experimental group for common carotid (COM), basilar (BAS), posterior communicating (PC), and middle cerebral (MCA) arteries. For reference, the line of identity is also indicated, and the arrows connecting the different points indicate the effects of maturation. Given this arrangement, a point below the line of identity indicates hypoxic depression of the sensitivity to norepinephrine, and conversely, a point above the line indicates hypoxic enhancement of norepinephrine sensitivity.

correlated. However, in the common carotid and middle cerebral arteries, no such correlation existed, which clearly indicated that other, probably presynaptic, mechanisms were involved, at least in these arteries.

Effects of altitude hypoxia on contractile responses to activation of the cerebrovascular adrenergic innervation

A common method used to study the pre-synaptic component of neurogenic vasoconstriction is to measure norepinephrine content using HPLC with electrochemical detection. The results of such studies conducted using arteries from normoxic and hypoxic term fetuses, and from normoxic and hypoxic non-pregnant adult sheep are shown in Figure 4. What we found was that hypoxia had a modest inhibitory effect on norepinephrine content in the arteries of the fetus, but had an augmenting effect on the arteries of the adult. At first glance, it appeared hard to reconcile these findings with the fact that hypoxia augmented neurogenic vasoconstriction in the fetus, but depressed it in the adult. However, it is important to remember that NE content is the product both the number of nerves in a tissue and the average content of NE per each nerve fiber. In addition, NE content is often lower in nerves that fire often, than in nerves that are generally quiescent¹. Thus, these results suggest that, compared to normoxic fetal arteries, hypoxic arteries have fewer nerve terminals or less NE per nerve terminal. In turn, the opposite must be true in the adult arteries; hypoxic arteries have more nerves or greater content per nerve. Overall, these content results demonstrate once again, that chronic hypoxia has opposite effects in fetal and adult arteries.

One way to better understand how hypoxia is affecting NE content is to independently measure nerve density. For adrenergic nerves, we can do this by quantifying cocaine-sensitive NE uptake which is a sensitive and selective measure of NE containing nerve terminals. Shown in Figure 5 are the results of such measurements in our four artery types. Based on these measurements, hypoxia appeared to increase

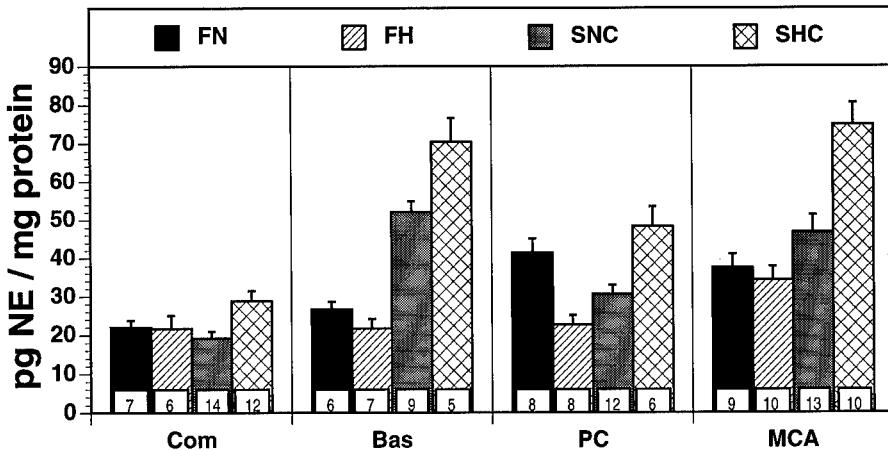


Figure 4 Effects of maturation and chronic hypoxia on norepinephrine content:
Shown here are the results of measurements of norepinephrine content using HPLC with electrochemical detection for common carotid (Com), basilar (Bas), posterior communicating (PC), and middle cerebral (MCA) arteries. The values are given relative to base-soluble protein and the error bars indicate standard errors for the number of animals indicated. The abbreviations FN, FH, SNC, and SHC indicate normoxic fetal, hypoxic fetal, normoxic adult, and hypoxic adult values, respectively.

nerve density in the larger common carotid and basilar arteries of both the fetus and adult. In the smaller and more peripheral arteries, most notably the middle cerebral

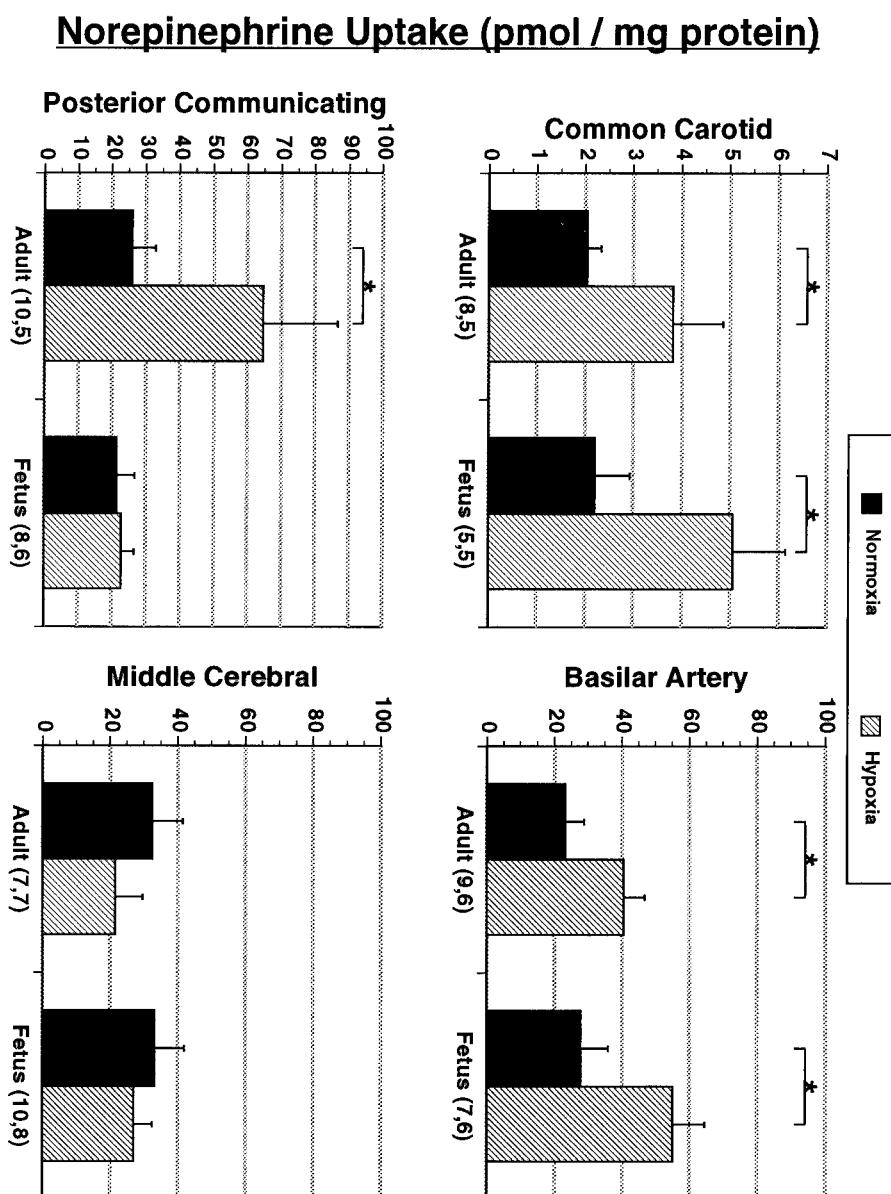


Figure 5 Effects of maturation and chronic hypoxia on norepinephrine uptake: Paired arteries were exposed to ^3H -norepinephrine with and without the presence of $0.1 \mu\text{M}$ cocaine. The differences between control and cocaine-treated arteries are shown above for our four basic artery types. Vertical error bars indicate standard errors for the numbers of animals indicated in parentheses. Asterisks indicate statistically significant differences between corresponding normoxic and hypoxic adult arteries at the $P<0.05$ level (ANOVA). Note that in the larger common carotid and basilar arteries, that hypoxia appears to increase nerve density in both fetal and adult arteries.

artery, hypoxia tended to decrease nerve density, although this effect was not quite statistically significant. Most importantly, hypoxia appeared to have a generally parallel effect on nerve density in both fetal and adult arteries, and thus density changes alone cannot explain the differential age-related effects of hypoxia on NE content. Combining these results with the NE content results, it then appears that chronic hypoxia is **decreasing** the content of NE per nerve, due possibly to **increased** nerve recruitment in fetal arteries, and in opposite fashion in adult arteries, is **increasing** the content of NE per nerve, due possibly to **decreased** nerve recruitment.

Effects of altitude hypoxia on cerebrovascular adrenergic-peptidergic co-transmission

Another factor that might also play a role in the effects of hypoxia on the contractile responses to nerve stimulation is the possible co-release of neuropeptide-Y, along with NE, from adrenergic nerve terminals. In many adrenergic nerves, NPY is co-localized with NE and released in varying ratios to NE in response to nerve stimulation⁸. To examine this possibility, we repeated our nerve stimulation experiments following depletion of NE vesicles from adrenergic nerve terminals by pretreatment with guanethidine. Following such treatment, any remaining tetrodotoxin-sensitive neurogenic responses can be attributed to NPY release. When arteries were treated with guanethidine and then stimulated at 8 Hz, the contractile responses (expressed relative to the maximum response to potassium) in the common carotid, basilar, posterior communicating, and middle cerebral arteries averaged 11.2 ± 2.0 , 2.2 ± 1.1 , 1.2 ± 0.7 , and 0.8 ± 0.5 % in normoxic adult arteries, 8.8 ± 2.0 , 0.6 ± 0.2 , 0.3 ± 0.1 , and 0.9 ± 0.4 % in hypoxic adult arteries, 0.0 ± 0.0 , 0.3 ± 0.2 , 0.0 ± 0.0 , and 0.2 ± 0.1 in normoxic fetal arteries, and 2.9 ± 1.9 , 0.7 ± 0.4 , 3.1 ± 1.6 , and 2.3 ± 1.1 in hypoxic fetal arteries. Thus, as before for responses to neuronally released norepinephrine, maturation enhanced normoxic responses to neuronally released NPY, and hypoxia generally depressed these responses in adult arteries and enhanced them in the fetal arteries. In other words, maturation increased the contribution from the NPY component under normoxic conditions, hypoxia generally depressed the NPY component in adult arteries, and enhanced it in fetal arteries. Given these results then, it seems appropriate to conclude that the presynaptic effects of hypoxia are similar for NE and NPY, and are opposite for fetuses and adults. In fetuses, hypoxia appears to decrease content per nerve due possibly to increased nerve activity. In adults, hypoxia appears to increase content per nerve due possibly to decreased nerve activity.

In summary then (Table 1), maturation increased responses to sympathetic nerve stimulation, and these responses were depressed by hypoxia in adult arteries, but were enhanced by hypoxia in fetal arteries. Maturation alone decreased sensitivity to NE in common carotid and middle cerebral arteries, but enhanced it in the arteries at the base of the brain. In basilar and posterior communicating arteries, hypoxia depressed post-synaptic NE sensitivity in the adult and enhanced it in the fetus, which can at least partially explain the effects of hypoxia on responses to nerve stimulation. In the common carotid and middle cerebral arteries, however, the effects of hypoxia on neurogenic vasoconstriction cannot be explained by changes in post-synaptic NE sensitivity, and thus other, probably presynaptic effects must be involved. Given the generally parallel effects of hypoxia on nerve density and NE content per nerve in fetal and adult arteries, the age-dependent differential effects of hypoxia on responses to sympathetic stimulation appear to involve age-related differences in nerve recruit-

		Common Carotid	Basilar Artery	Posterior Communicating	Middle Cerebral
Sympathetic Responses	Maturation Effect	↑	↑	↑	↑
	Chronic Hypoxia	↓↑	↓↑	↓↑	↓↑
Norepinephrine Sensitivity	Maturation Effect	↓	↑	↑	↓
	Chronic Hypoxia	↓--	↓↑	↓↑	--↓
SANS Innervation	Maturation Effect	—	↓	↑	↓
	Chronic Hypoxia	↑↑	↑↑	↑↑	↓↓
Non-SANS Responses	Maturation Effect	↑	↑	↑	↑
	Chronic Hypoxia	--↑	↓↑	↓↑	--↑

Table 1: Contractility Summary

Summarized above are the effects of maturation and chronic hypoxia on responses to sympathetic nerve stimulation, norepinephrine sensitivity (as indicated by pD_2 values), sympathetic innervation, and the peptidergic component of contractile response to adrenergic nerve stimulation (defined as the guanethidine-resistant component of response to transmural nerve stimulation). The index of sympathetic innervation indicated here reflects the ratio of uptake to content, which is an index of the NE content per nerve. The effects of maturation were determined by comparing normoxic fetal and normoxic adult characteristics. The effects of chronic hypoxia were determined by comparing age-matched normoxic and hypoxic characteristics. The effects of chronic hypoxia are indicated for adults by the leftmost arrows or dashes in each pair, and the rightmost arrows or dashes for fetuses. Dashes indicate no effect.

ment, due possibly to differences in factors such as quantal release or the thresholds for activation. These age-related differences do not seem to be due to differences in NPY release from adrenergic nerve terminals, as patterns of NPY release generally follow those observed for NE release.

Effects of altitude hypoxia on endothelium-dependent cerebral vasodilator responses

Overall, hypoxia has a generally depressant effect on contractility, when measured in potassium- or amine-contracted arteries. Opposite age-dependent effects of hypoxia on contractility are most evident when responses to adrenergic nerve stimulation are examined. Interestingly, such differences are generally not observed when responses to vasodilator innervation are examined. We have performed such experiments using capsaicin to release vasodilator peptides such as substance-P from vascular nerve terminals, but in these experiments, the effects of hypoxia are generally depressive in both fetal and adult arteries. Apart from nerve-mediated responses, vasorelaxation can also be produced by several other mechanisms, the most important of which is endothelium-dependent relaxation.

In overview, the luminal surface of the endothelium contains a variety of receptors, activation of which stimulates the enzyme nitric oxide synthetase, which in turn synthesizes nitric oxide from L-arginine⁷. This nitric oxide can then diffuse into the adjacent vascular smooth muscle where it can activate guanylate cyclase to synthesize cGMP which then promotes vasorelaxation through activation of G-kinase and subsequent phosphorylation of an unknown protein, possibly Ca-ATPase in either the sarcolemma or sarcoplasmic reticulum or a membrane potassium channel. In addition to agonists which activate the luminal endothelial receptors, endothelial NO-synthetases can also be activated in a receptor-independent fashion through the action of A23187, a calcium ionophore which facilitates the entry of activating levels of calcium into the endothelial cytoplasm. At the vascular level, we can also stimulate guanylate cyclase directly via the use of nitric oxide releasing agents such as S-nitroso-N-acetyl-penicillamine or nitroglycerin².

When 10 μ M acetylcholine was added to arteries with an intact endothelium, the relaxation responses varied markedly with both age and artery type (Fig. 6). Endothelium-dependent responses to acetylcholine improved with maturation in the common carotid, but worsened with maturation in the basilar, posterior communicating, and middle cerebral arteries. Interestingly, chronic hypoxia had opposite effects on common carotid responses to acetylcholine in fetal and adult arteries. In the adult, hypoxia attenuated these responses, but augmented them in the fetus, and a similar pattern was also observed in the middle cerebral. Effects of hypoxia were also opposite in the posterior communicating, where they were augmented in the adult and depressed in the fetus. Thus once again, we appeared to have age-dependent differential effects of hypoxia in adult and fetal cerebral arteries.

One way to examine how hypoxia produced these effects was to compare the acetylcholine responses to responses to A23187, keeping in mind that responses to A23187 are receptor-independent whereas those to acetylcholine are not. Thus differences in responses to acetylcholine and A23187 can be attributed to differences in acetylcholine receptor density. When we examined responses to A23187, we found a pattern of effects quite different than observed for acetylcholine responses (Fig. 7). Maturation alone generally had little effect, suggesting that the maturation effects observed with acetylcholine were due largely to age-related changes in acetylcholine receptor density, distribution, and/or affinity. Chronic hypoxia, however, still produced opposite effects in fetal and adult arteries. In adult arteries, A23187 responses were enhanced by hypoxia, but were consistently depressed by hypoxia in fetal arteries. This pattern of effects then suggests that hypoxia somehow enhanced endothelium-dependent vasodilatation in adult arteries, but depressed it in fetal arteries. In addition, these results also suggest that the age- and artery-specific effects of hypoxia on responses to acetylcholine further involved changes in acetylcholine receptor density and/or coupling to endothelium-mediated relaxation.

Effects of altitude hypoxia on endothelium-independent cerebral vasodilator responses

Just as responses to acetylcholine are affected by changes in the overall ability of the endothelium to promote relaxation, responses to A23187 are similarly affected by changes in the ability of the underlying vascular smooth muscle to respond to nitric oxide released from the endothelium. To examine how vascular sensitivity to nitric oxide changes during maturation and chronic hypoxia, we examined relaxation re-

sponses to S-nitroso-N-acetyl-penicillamine, otherwise known as SNAP, which releases nitric oxide in a mole-to-mole ratio upon hydration². As for responses to A23187, the effects of maturation on responses to SNAP were minimal⁶. In addition, hypoxia attenuated responses to 10 μ M SNAP in common carotid, basilar, posterior commu-

Percent Relaxation to 10 μ M Acetylcholine

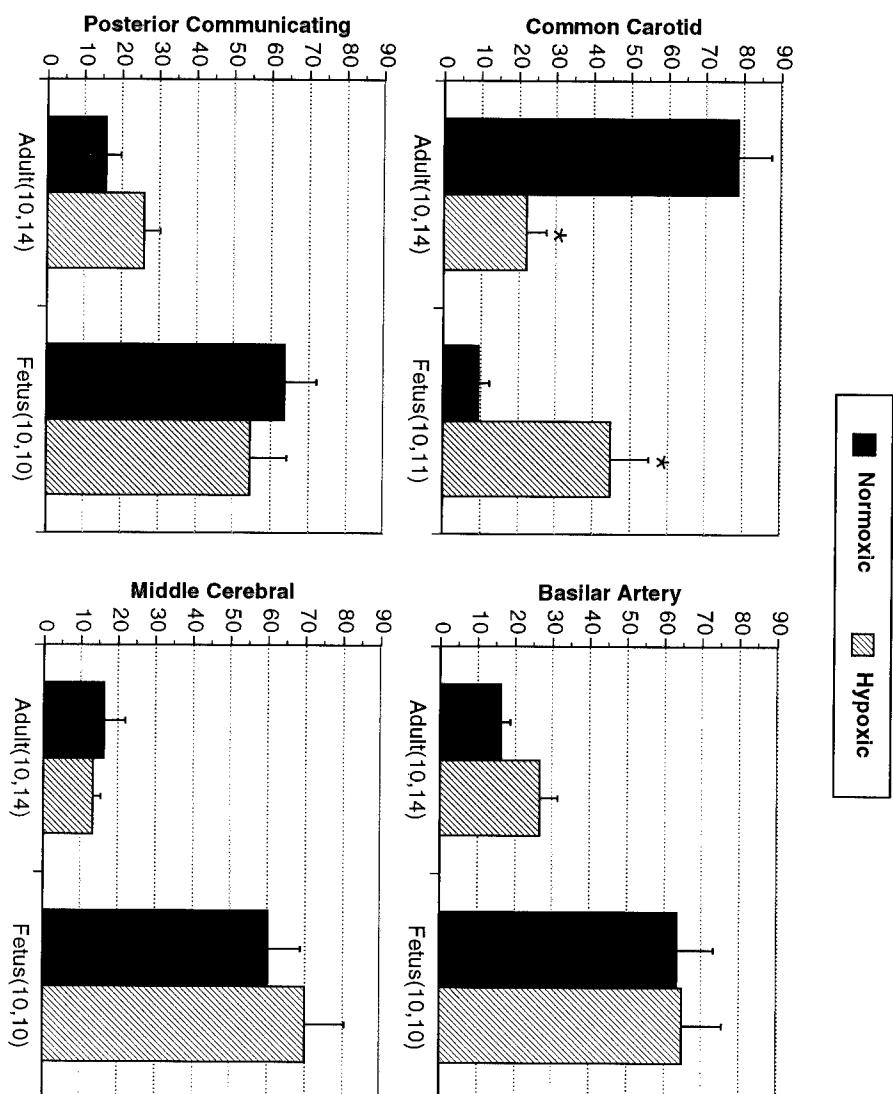


Figure 6 Effects of maturation and chronic hypoxia on relaxation responses to acetylcholine: Shown here are the averaged responses of arteries, pre-contracted with 1 μ M serotonin, to 10 μ M acetylcholine. The vertical error bars indicate standard errors for the number of animals indicated. Asterisks indicate statistically significant differences between fetal and adult arteries at the P<0.05 level (ANOVA).

nicating, and middle cerebral arteries by -3.0, -12.4, -9.8, and -14.1% in the adult and by -9.3, -11.0, -14.7, and -13.1% in the fetus. Thus the general effects of hypoxia were consistent across both age groups. Hypoxia mildly attenuated the ability of all arteries to respond to nitric oxide, and this modest effect was significant only by ANOVA across all arteries of both age groups. Most importantly, this pattern sug-

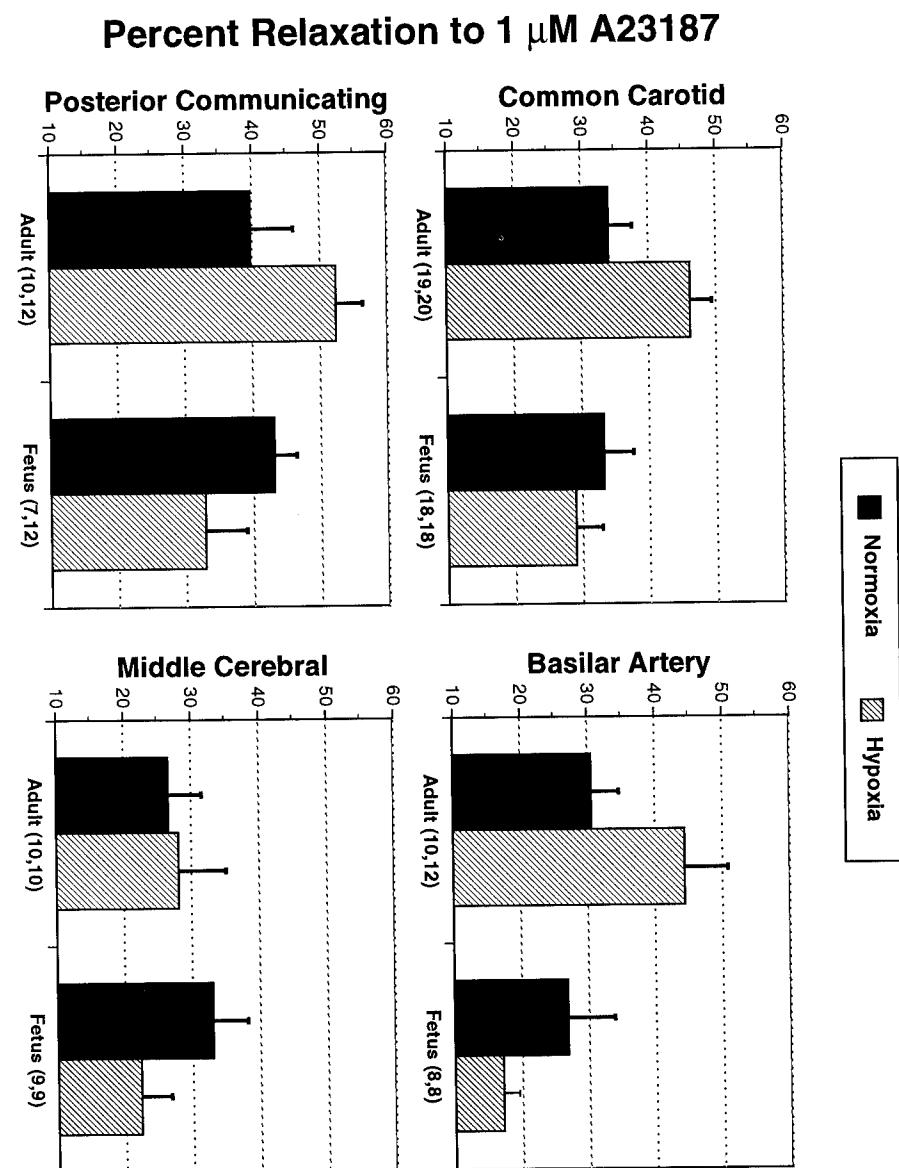


Figure 7 Effects of maturation and chronic hypoxia on relaxation responses to A23187. Shown here are the averaged responses of arteries, pre-contracted with 1 μ M serotonin, to 1 μ M A23187, a calcium ionophore which stimulates endothelium-dependent relaxation independent of membrane receptor mechanisms. The vertical error bars indicate standard errors for the number of animals indicated.

gests that the changes in responses to A23187 observed with hypoxia must have been due to changes at the endothelial level.

One great advantage of knowing how maturation and hypoxia affect responses to SNAP is that it allows for the correction of other responses for any changes in vascular sensitivity to nitric oxide. By correcting responses to A23187 relative to those to SNAP, it is thus possible to examine the effects of hypoxia on the ability of the endothelium to release nitric oxide. Using this approach (Fig. 8), it is quite evident that hypoxia tended to modestly depress endothelial vasodilator capacity in the fetus, although these effects were not individually significant. In contrast, hypoxia significantly augmented endothelial vasodilator capacity in all adult arteries. Once again, the effects of hypoxia were opposite in fetal and adult cerebral arteries.

In summary (Table 2), maturation enhanced responses to acetylcholine in the common carotid arteries, but depressed these responses in the intracranial arteries. Hypoxia depressed adult carotid responses to acetylcholine and enhanced them in arteries at the base of the brain. In fetal arteries, hypoxia had no significant effects except in the common carotid where responses were enhanced. Because maturation alone had no significant effects on responses to A23187, a receptor-independent activator of the endothelium, endothelial capacity probably changed little with age. Thus, the age-related changes observed with acetylcholine were due to maturational increases or decreases in acetylcholine receptor density and/or affinity. Hypoxia however, significantly enhanced A23187 responses in adult arteries and had no effect on these responses in fetal arteries. This finding in turn suggests that hypoxia probably increased acetylcholine receptor density or affinity in fetal carotid arteries, and markedly decreased these variables in the adult common carotid. At the vascular level, the ability to relax in response to nitric oxide decreased slightly in response to either maturation or chronic hypoxia. But even when responses to A23187 were corrected for these effects, age-dependent differential effects of chronic hypoxia on endothelial vasodilator capacity were still significant. Hypoxia enhanced endothelial vasodilator capacity in the adult arteries, and had a mild depressive effect on the fetal arteries.

Conclusions

Altogether the main significant effect of chronic hypoxia on cerebral artery structure and composition was to increase protein content, and this effect was observed in both fetal and adult cerebral arteries. Hypoxia also depressed contractile responses to potassium and exogenous amines, and this effect was again observed in both fetal and adult arteries. When these responses were corrected for age-related differences in cross-sectional area, the hypoxic decreases in contractility were significant only in the fetal arteries. Opposing differences between fetal and adult artery responses to hypoxia became even more evident when we examined responses to adrenergic nerve stimulation. There, hypoxia enhanced fetal responses but depressed those in adult arteries. Some of these age-related differences were due to enhancement of NE sensitivity in fetal arteries and depressions of NE sensitivity in adult arteries, but presynaptic effects were also involved. Presynaptically, hypoxia tended to increase innervation density and neuronal NE content, but tended to depress nerve recruitment in adult arteries and enhanced it in fetal arteries. Furthermore, these presynaptic effects were similar for both the norepinephrine and the neuropeptide Y contained within adrenergic nerve terminals. Although hypoxia tended to depress neurogenic vasodilation in both fetal and adult arteries, it had opposite effects on endothelium dependent

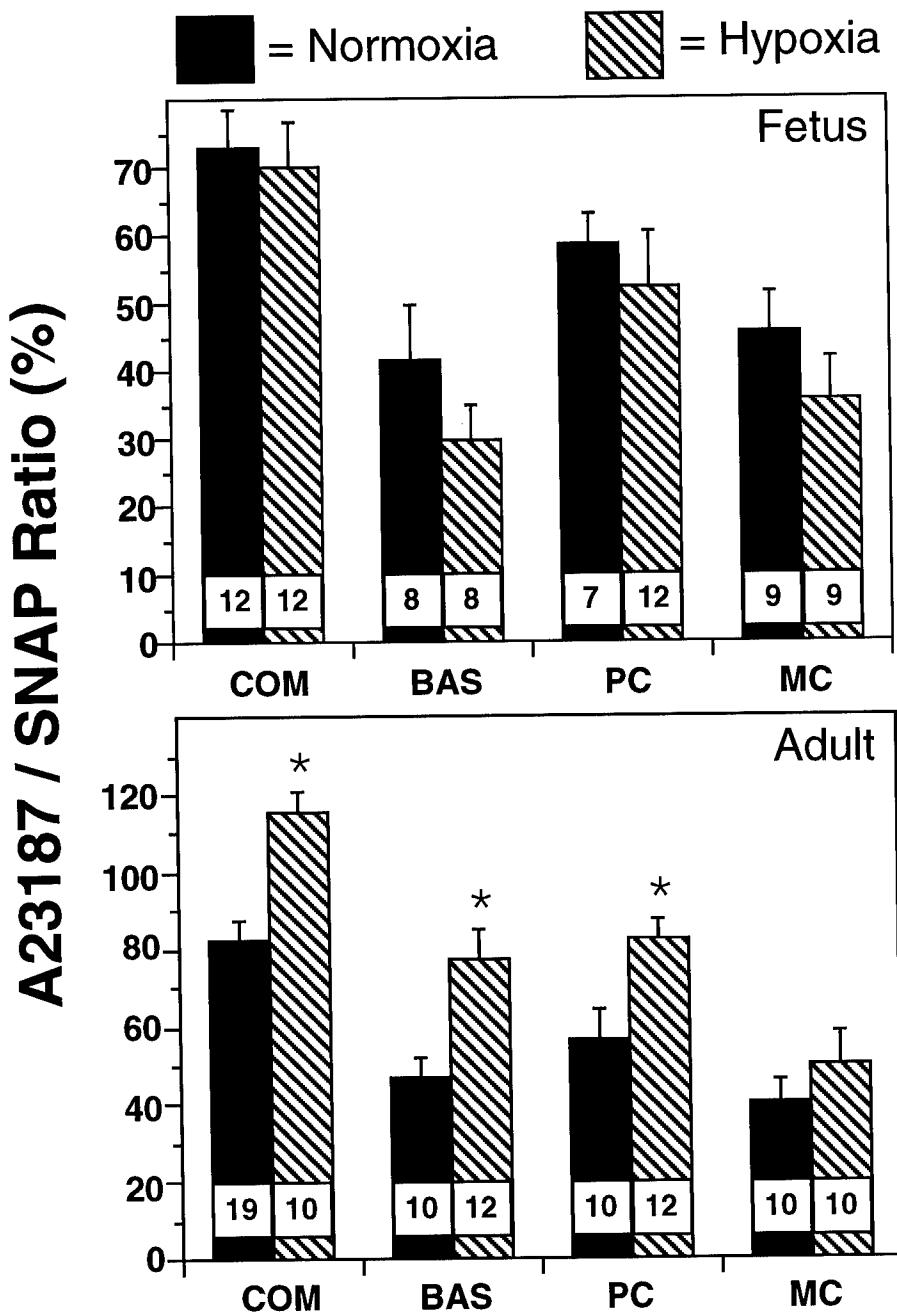


Figure 8 Effects of maturation and chronic hypoxia on the A23/SNAP ratio:
 Shown in this figure are the A23187 data shown in Figure 7, expressed as A23187/SNAP ratios and thus corrected for changes in vascular sensitivity to nitric oxide for common carotid (COM), basilar (BAS), posterior communicating (PC), and middle cerebral (MC) arteries. The vertical error bars indicate standard errors for the number of animals indicated. Asterisks indicate statistically significant effects of hypoxia at the $P<0.05$ level.

	Common Carotid	Basilar Artery	Posterior Communicating	Middle Cerebral
Acetylcholine Responses	Maturation Effect	↑	↑	↑
	Chronic Hypoxia	---	---	---
A23187 Responses	Maturation Effect	↑	—	—
	Chronic Hypoxia	↑↑	↑↑	↑↑
SNAP Responses	Maturation Effect	↓	↓	—
	Chronic Hypoxia	---	↓—	---

Table 2: Vasodilatation Summary

Summarized above are the effects of maturation and chronic hypoxia on responses to acetylcholine, A23187, and the nitric oxide releasing agent, S-nitroso-N-acetyl-penicillamine. The symbols above are used as described for Table 1.

vasodilatation in the two age groups. It enhanced endothelial vasodilator capacity in adult arteries, and depressed it in fetal arteries. Superimposed on these effects were artery and age-specific changes in acetylcholine receptor density and distribution. Hypoxia had only a mild depressive effect on vascular responses to directly applied nitric oxide, and these effects were equivalent in both fetal and adult arteries.

The take-home message then is that hypoxia does indeed have multiple important effects on fetal cerebrovascular development, and also on cerebral artery function in adults. Many of the effects of hypoxia are parallel in fetuses and adults, such as those on protein content, non-neurogenic contractile responses, adrenergic nerve density, neurogenic vasodilator responses, and responses to nitric oxide. In contrast, hypoxia has opposite effects on fetal and adult artery neurogenic vasoconstriction and endothelium-dependent vasodilatation. How are these different effects of hypoxia produced? Where is hypoxia sensed and how are the long-term responses to hypoxia coordinated? Most importantly, are these responses advantageous and representative of successful compensation in both the fetus and the adult? Or do these responses reflect any pathophysiology that may contribute to the greater cerebrovascular morbidity observed in both newborns and adults at altitude? Many questions remain, and with luck some answers to these and other questions should be forthcoming in the next few years, and hopefully will be presented at a future International Hypoxia Symposium.

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CHAPTER 12

FETAL CARDIOVASCULAR DEVELOPMENT AT ALTITUDE

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ABSTRACT

Because the fetal heart is normally functioning near its maximum capability, it is very sensitive to periods of hypoxemia. We have characterized the fetal sheep heart response to long-term (110 days) high altitude (3,820m) hypoxemia and found a decrease in total cardiac output that is almost entirely due to a decrease in right ventricular output. The fetus does redistribute the decreased cardiac output to maintain normal blood flow to the heart and brain at the expense of the body. In the calcium pathway for initiation of contraction, there was no change in sarcolemmal L-type calcium channel number in either ventricle of high altitude fetuses, but an increase was noted in sarcoplasmic reticulum calcium channels in the right ventricle. The right ventricle of high altitude fetuses also showed a greater dependence on calcium stored in the sarcoplasmic reticulum for initiation of contraction. β -adrenergic receptor enhancement of myocardial contractility was reduced in the left ventricle of intact high altitude fetuses, but no change was seen in the right ventricle. However, in isolated papillary muscle a reduction in response to isoproterenol was noted in both ventricles. An increase in β -adrenergic receptor number was found in the right, but not left, ventricle of high altitude fetuses, suggesting alterations in the coupling of receptor to intracellular second messengers. Energy production in high altitude fetal hearts appears to be enhanced as judged by increases in lactate dehydrogenase and citrate synthase, enzymes associated with the metabolism of lactate, the primary fuel of the fetal heart. Papillary muscle from high altitude fetal hearts also demonstrated the ability to sustain stronger contractions in hypoxic conditions compared to control hearts. Clarification of the intracellular mechanisms involved in the alteration of cardiac function seen in high altitude fetal hearts will entail further studies of the calcium pathway responsible for the initiation of contraction, the augmentation of contractility by the β -adrenergic receptor system, and of energy production.

Introduction

Late gestation is a critical period in the development of the mammalian heart and cardiovascular system. Key developmental changes occur in coronary blood vessel growth, arrangement, autonomic innervation, and regulation of vascular tone. Key changes are also occurring in myocardial myocyte number and size, myofilament content and organization, myosin isozymes, sarcoplasmic reticulum content and function, autonomic innervation and its coupling to effector pathways, and energy pro-

duction. Perturbations, such as bouts of intrauterine hypoxemia, have been hypothesized as etiologic factors causing delayed myocardial maturation and increased perinatal mortality and morbidity. However, little evidence exists to support this hypothesis. Therefore, we undertook an examination of the effects of long-term intrauterine hypoxemia on fetal cardiac function and maturation.

When function of the fetal heart is described by the relationship between output, stroke volume, or stroke work and atrial or end diastolic pressure, the normal fetal heart has been found to operate near the plateau (or maximum) of its function curve and to be very sensitive to increases in afterload (arterial pressure)^{11,12,13,18,27}. Based on these findings the fetal heart might be expected to be very sensitive to hypoxemia. However, during acute (30 min to 2 h) moderate hypoxemia fetal cardiac output does not change dramatically, either remaining constant or falling an insignificant amount^{7,8,22}. The fetus does respond to the acute hypoxemia with increased arterial pressure⁷, bradycardia^{3,7}, increased blood flow to the heart and brain at the expense of other organs^{14,22,26}, and increased production of several hormones which support the cardiovascular system, including catecholamines, vasopressin, renin, and erythropoietin^{6,21,23}. However, whether the fetus can maintain its cardiac function and what mechanisms might be involved during longer periods of hypoxemia has not been studied.

Short-Term Hypoxemia

We have carried out a number of studies of the developing fetal cardiovascular system response to different periods of hypoxemia. We initially subjected pregnant ewes and their fetuses to 14 days of hypoxemia (maternal PO₂ \leq 60 Torr) beginning on day 120 of gestation (term = 145 days) and characterized both maternal and fetal cardiovascular and endocrine responses. The pregnant ewes responded with a small increase in hemoglobin concentration by day 2 of the hypoxic period which lasted throughout the 2 weeks of study. Maternal erythropoietin levels were doubled by 24 h, but quickly returned to near normal levels for the remainder of the study. Maternal mean arterial pressure, cardiac output, heart rate, blood volume, body weight, uterine blood flow, uteroplacental O₂ uptake, and the concentrations of glucose, catecholamines, and cortisol remained relatively constant²⁰. Fetal erythropoietin levels rose to 5 times normal by 24 h, and then returned to levels just slightly above control for the remainder of the study. This was followed by a gradual rise in fetal hemoglobin from 10 to 13 g/dl by day 7, where it remained. Fetal heart rate did not change, but fetal arterial pressure and epinephrine levels were moderately elevated throughout the study period¹⁹. Fetal right ventricular output was significantly depressed by day 3, whereas left ventricular output was not reduced until day 7. By day 14 there was a 30-40% decrease in total fetal cardiac output (the sum of right and left ventricular outputs). At 14 days of hypoxemia the sensitivity of the left ventricle to increases in afterload (arterial pressure) was unchanged, but the right ventricle had become less sensitive to increased afterload^{1,16}.

Long-Term Hypoxia

We then carried out a series of fetal cardiovascular studies for a longer period of hypoxemia in pregnant ewes exposed to 100-110 days of hypoxemia beginning on day 30 of gestation, which form the main basis for this report. To carry out these studies pregnant sheep were transported to the White Mountain Research Station, CA (3,820m) at 30 days gestation, where they were housed until delivery to Loma Linda

University for study. Upon arrival at Loma Linda University a nonocclusive maternal tracheal catheter was implanted and nitrogen infused through this catheter to maintain the maternal arterial PO_2 the same as at high altitude during the further course of experimentation. Table 1 shows the maternal and fetal blood gases and hemoglobin of these animals after their residence at high altitude. The 17-18% decrease in fetal arterial PO_2 would be classified as mild to moderate fetal hypoxemia, yet it produced an ~20% increase in fetal hemoglobin levels.

Table 1.

Maternal and fetal blood gases at high altitude.

	Control	High Altitude
Mother		
PO_2 (Torr)	102.1 \pm 1.9	64.2 \pm 2.4*
PCO_2 (Torr)	35.2 \pm 0.9	28.9 \pm 2.4*
pH	7.44 \pm 0.01	7.46 \pm 0.01
[Hb] (g/dl)	8.7 \pm 0.3	10.5 \pm 0.4*
Fetus		
PO_2 (Torr)	23.3 \pm 0.5	19.3 \pm 0.8*
PCO_2 (Torr)	48.9 \pm 1.2	39.9 \pm 0.9*
pH	7.33 \pm 0.01	7.35 \pm 0.01
[Hb] (g/dl)	9.6 \pm 0.2	11.6 \pm 0.4*

* significantly different from control, $p<0.05$

Cardiovascular Responses

Following this length and level of hypoxemia total fetal cardiac output was significantly decreased (-24 %) compared to normoxic fetuses (Fig. 1). This was brought about by a significant reduction in fetal right ventricular output (-34 %), with little change in left ventricular output. Thus, long-term hypoxemia beginning early in gestation (30 days) resulted in changes in ventricular outputs somewhat different than 14 days of hypoxemia beginning late in gestation (120 days), during which both ventricular outputs were decreased. We do not know in the longer exposure studies if left ventricular output decreased and subsequently returned to normal or if it never decreased. In spite of this decreased total cardiac output, these long-term hypoxic fetuses maintained a normal blood flow to the heart and brain (Fig. 2) at the expense of a greater than 50% reduction in blood flow to skeletal muscle, skin, and bone (carcass)¹⁵. Thus, a blood flow pattern exists in these fetuses which would be consistent with the production of asymmetric growth retardation.

Mechanisms of Alterations in Cardiac Contractility

Subsequently, we began to examine several factors involved in cardiac contractility (Fig. 3) which might elucidate the mechanisms involved in the reduction in cardiac function observed in the long-term hypoxic fetuses. These areas included: 1) the calcium pathway involving the calcium induced calcium release mechanism responsible for the initiation of contraction, 2) the augmentation of contractility by the

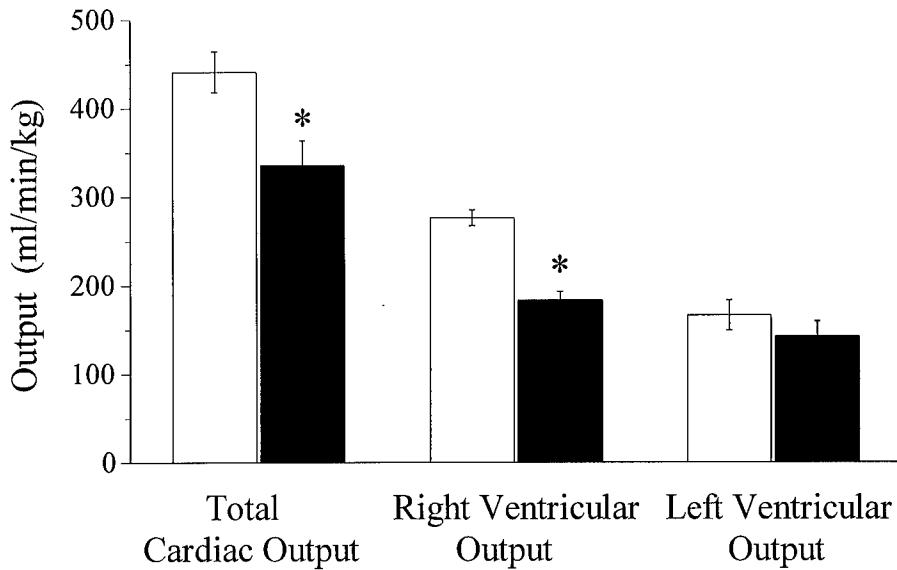


Figure 1 Right and left ventricular output and total cardiac output in control (open bars) and high altitude (black bars) fetuses. * significantly different from control, $p<0.05$.

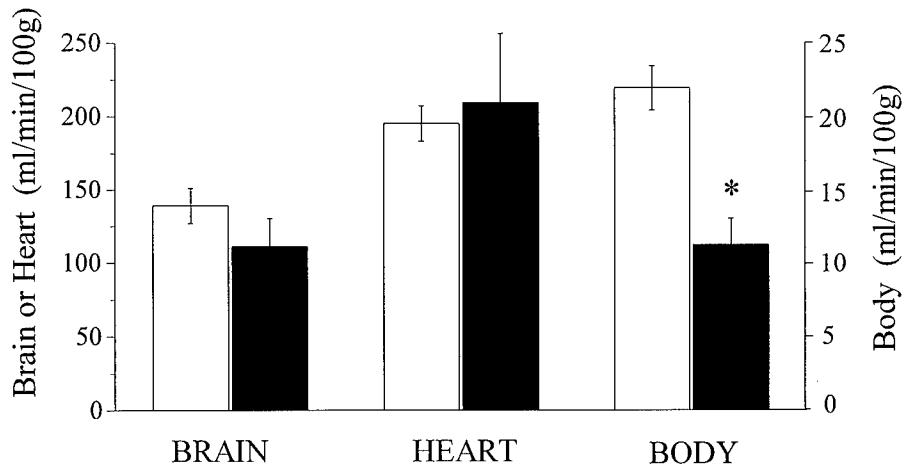


Figure 2 Blood flows to the brain, heart, and body in control (open bars) and high altitude (black bars) fetuses. * significantly different from control, $p<0.05$.

β -adrenergic receptor system, and 3) the energy production pathways involved in formation of ATP.

Calcium Pathway

To initiate a contraction, a cardiac action potential propagated over the myocardial cell surface causes the opening of L-type calcium channels located in the T-

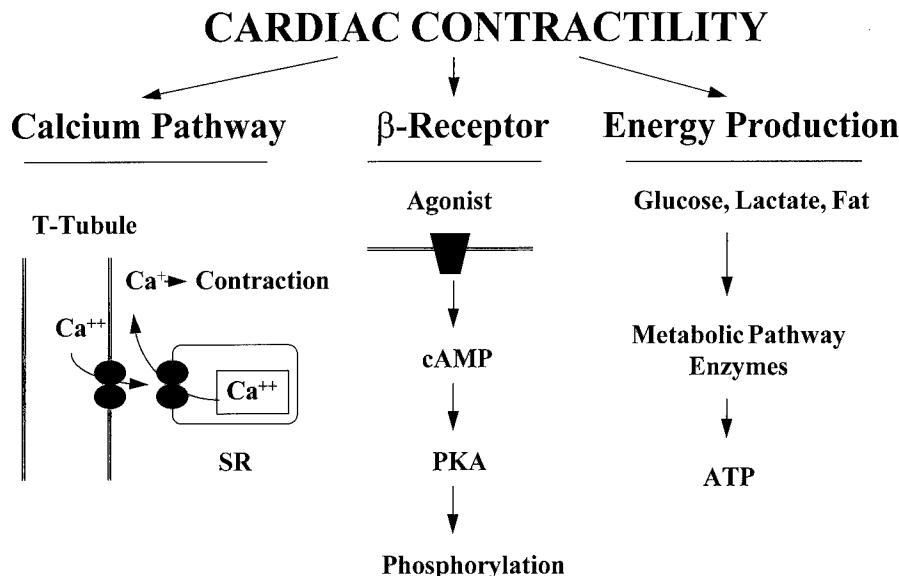


Figure 3 Schematic of the calcium, β -adrenergic receptor, and energy production pathways involved in initiation and maintenance of cardiac contractility.

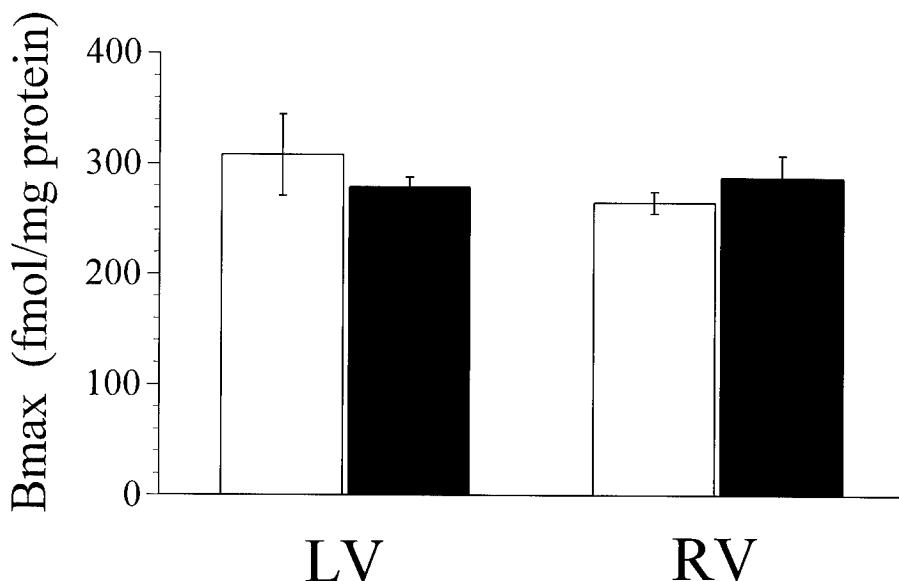


Figure 4 Number (Bmax) of L-type calcium channels in the left (LV) and right (RV) ventricles of control (open bars) and high altitude (black bars) fetal hearts.

tubule region of the sarcolemma. Calcium enters the cell through these channels where much of it binds to specialized calcium channels located on the sarcoplasmic reticulum (SR). The binding of calcium to the SR calcium channels induces the release of

calcium stored in the sarcoplasmic reticulum (calcium induced calcium release). Thus, calcium from both sources (extracellular and sarcoplasmic reticulum) can then diffuse and bind to troponin C, resulting in the initiation of contraction.

We have examined the effects of high altitude on the number of both L-type and SR calcium channels and also on the relative importance of calcium induced calcium release. The number of L-type calcium channels located in the T-tubular region of the sarcolemma was unchanged in the high altitude fetuses (Fig. 4). However, the number of SR calcium channels was increased in the right ventricle of high altitude fetuses (Fig. 5), although it remained unchanged in the left ventricle.

To test the relative importance of the calcium-induced calcium release mechanism, we blocked the release of calcium from the sarcoplasmic reticulum by administering ryanodine to papillary muscle strips maintained and stimulated in an isolated bath system. A reduction in maximum developed tension (Tmax) in the papillary muscles following ryanodine would indicate the minimum participation in contraction of calcium stored in the sarcoplasmic reticulum. In the left ventricle (Fig. 6) ryanodine produced an approximate 55% reduction in maximum developed tension in both the control and high altitude fetuses. However, in the right ventricle ryanodine produced an ~70% decrease in Tmax in the high altitude fetuses compared to only a 50% decrease in the control fetuses. Thus, in the left ventricle, which demonstrated no change in output in the high altitude fetuses (Fig. 1), there was also no change in the calcium pathway for initiation of contraction. The right ventricle of high altitude fetuses showed a greater dependence on calcium stored in the sarcoplasmic reticulum for initiation of contraction, as indicated by a greater number of SR calcium channels (Fig. 5) and a greater reduction in contractility when calcium release from the SR was

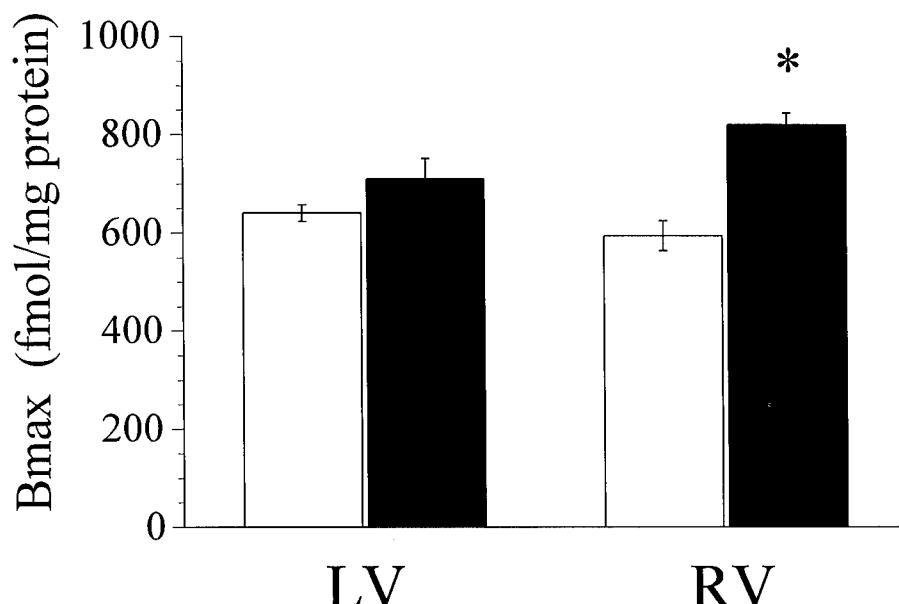


Figure 5 Number (Bmax) of calcium channels on the sarcoplasmic reticulum of left (LV) and right (RV) ventricles of control (open bars) and high altitude (black bars) fetal hearts. * significantly different from control, $p < 0.05$.

blocked (Fig. 6). This response would not explain the reduction in right ventricular output observed in the high altitude fetuses (Fig. 1), but might indicate an adaptive response toward a possibly more mature contractile process in an attempt to overcome the reduced output. Therefore, the myocardial mechanism responsible for the reduction in right ventricular output in the high altitude fetuses remains unexplained. Further investigation might focus on changes in the myocardial action potential, sensitivity of troponin to calcium, or changes in myosin isoforms and isozymes.

β -Adrenergic Receptor Pathway

The β -adrenergic receptor system can enhance myocardial contractility after occupation of the receptor by an agonist (Fig. 3). This is accomplished mainly by a resulting increase in cAMP and protein kinase A (PKA), resulting in phosphorylation of L-type calcium channels and an increased entry of calcium during a myocardial action potential. Evidence also suggests the direct stimulation of calcium movement through L-type calcium channels by a stimulatory G_s protein. We have examined the effects of the β -adrenergic receptor pathway in both the high altitude and control intact fetus¹⁷ by measuring the right and left ventricular output response to an isoproterenol dose response curve (Fig. 7). We found no difference between the high altitude and control right ventricular response to isoproterenol. However, the high altitude left ventricular response was diminished compared to the control left ventricle. We also examined the response to isoproterenol in papillary muscle isolated from both high altitude and control fetuses (Fig. 8). As shown by the measurement of maximum developed tension (Tmax) to a maximum dose of isoproterenol, there was a decrement in both right and left ventricular responses to β -adrenergic receptor stimu-

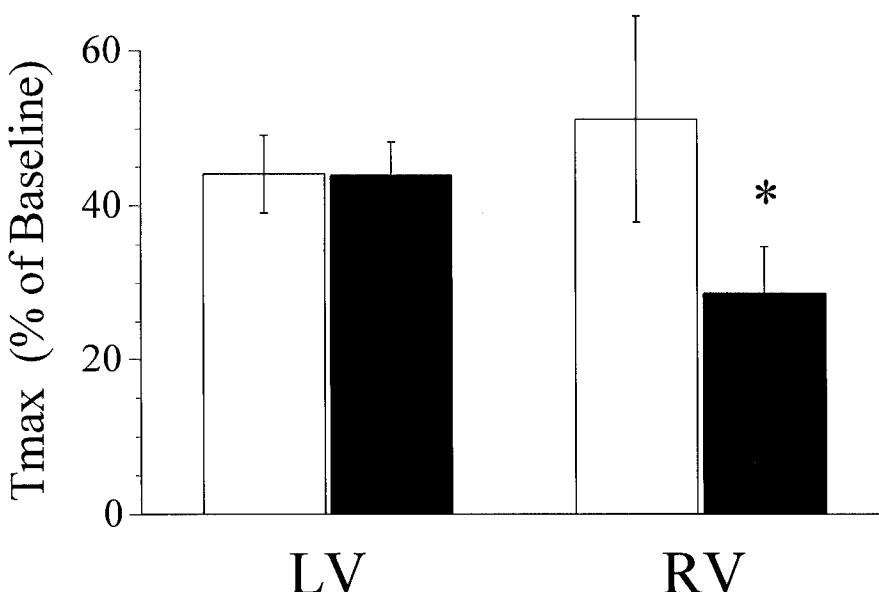


Figure 6 Reduction in maximum developed tension (Tmax) of isolated papillary muscles by the blockade of calcium release from sarcoplasmic reticulum with ryanodine in control (open bars) and high altitude (black bars) fetal hearts. * significantly different from control, $p < 0.05$.

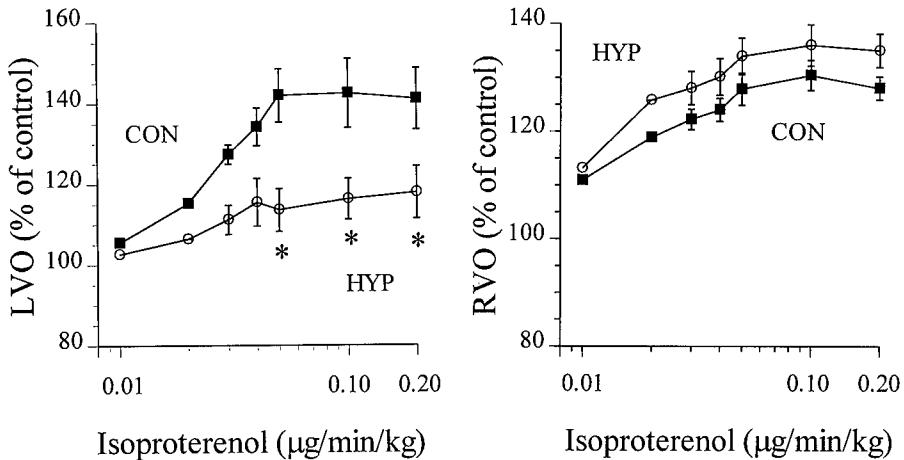


Figure 7 Effect of β -adrenergic receptor stimulation with isoproterenol on left (LVO) and right (RVO) ventricular outputs of control (closed squares - CON) and high altitude (open circles - HYP) fetuses. * significantly different from control, $p < 0.05$.

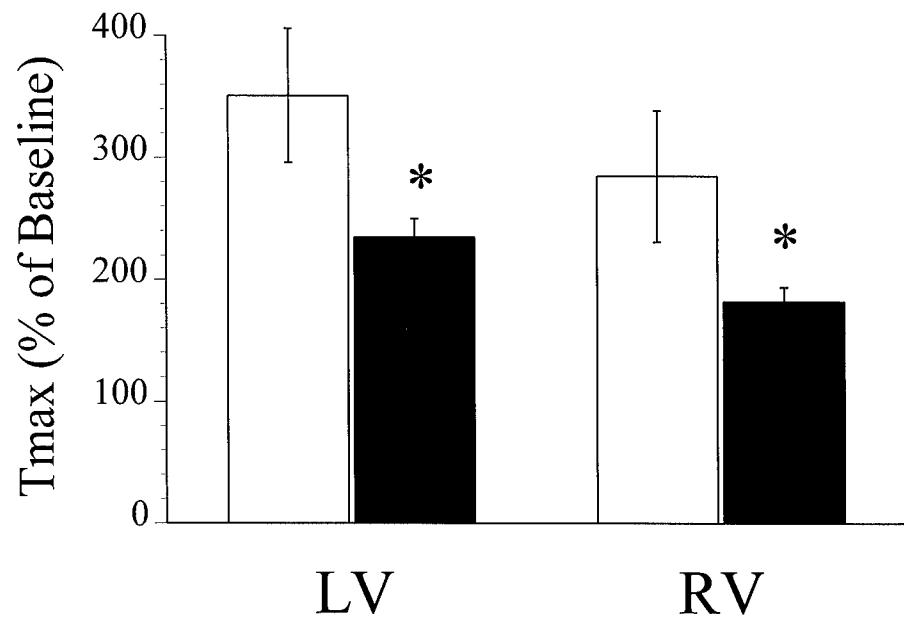


Figure 8 Effect of isoproterenol on the maximum developed tension (Tmax) of papillary muscle isolated from the left (LV) and right (RV) ventricles of control (open bars) and high altitude (black bars) fetuses. * significantly different from control, $p < 0.05$.

lation. We are not sure of the reason for the difference in the isolated papillary muscle response compared to the intact fetal heart response to isoproterenol. We have measured β -adrenergic receptor number (Fig. 9), finding an increased abundance of β -

adrenergic receptors in the right ventricle of the high altitude fetuses, but no difference in the left ventricle.

Others have reported a decrease in β -adrenergic receptor number in both ventricles of adult rats²⁸, newborn rabbits², and cultured chick heart cells²⁴ exposed to long-term hypoxemia. However, Mader²³ reported no such decrease in 20 month old rats and in newborn lambs^{3,4} and young (3 month) rats²³ a reduction in β -adrenergic receptor number was seen only in the left ventricle.

The reduction in functional response to isoproterenol we noted in the left ventricle (Fig. 7 and 8) in the face of no change in β -adrenergic (Fig. 9) receptor number suggests alterations in the coupling of receptors to intracellular second messengers. This is also true of the right ventricle which exhibited either no change in response to isoproterenol in the intact heart (Fig. 7) or a decrease in the isolated papillary muscle (Fig. 8) in the face of an increased number of receptors (Fig. 9). Bernstein³ has found in the left ventricle of chronically hypoxic newborn lambs that isoproterenol-stimulated cAMP production was reduced compared to controls, but that forskolin-stimulated cAMP production in those ventricle was not different from control, suggesting a reduction in receptor coupling to adenylate cyclase in these hypoxic newborn left ventricles.

Clarification of the alterations in β -adrenergic receptor function in high altitude fetal hearts must await measurement of linking between the receptor and intracellular messengers. We are in the process of measuring cAMP production in response to β -adrenergic receptor stimulation, as well as to direct stimulation by forskolin, to examine the linking of the receptor to the adenylate cyclase system. Further studies will focus on the increases in the intracellular binding of protein kinase A in response to β -adrenergic receptor stimulation and to direct stimulation with dibutyryl cAMP.

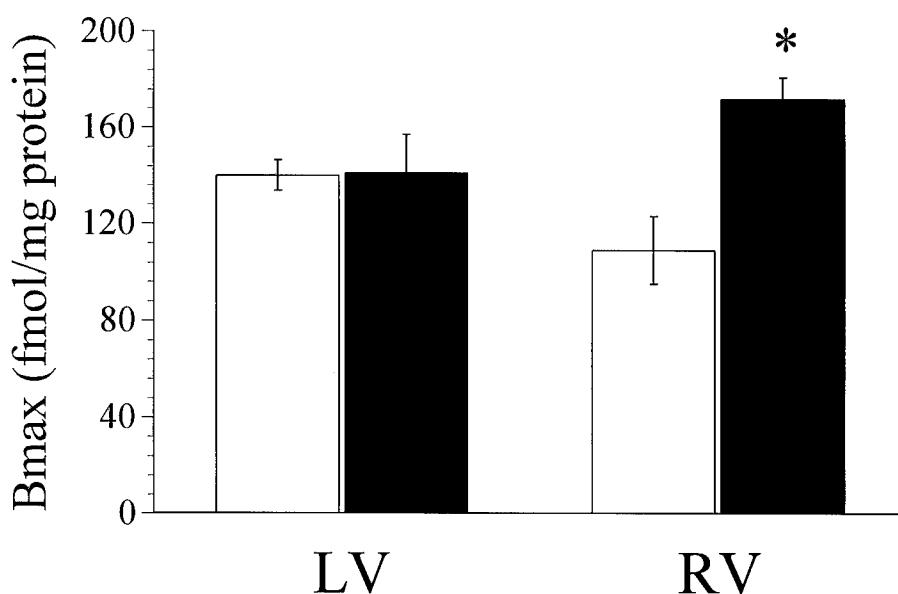


Figure 9 Number (Bmax) of β -adrenergic receptors in the left (LV) and right (RV) ventricles of control (open bars) and high altitude (black bars) fetuses. * significantly different from control, $p < 0.05$.

Energy Production

All contractile processes in the heart are sustained by the production of ATP as the energy source for all intracellular processes. We hypothesized that high altitude fetal hearts would develop an enhanced ability to produce ATP. We have begun to test this hypothesis by measuring key regulatory metabolic enzymes involved in energy production. Because the fetal heart utilizes glucose and lactate for energy production to the exclusion of free fatty acids^{9,10}, we chose to initially examine pyruvate kinase as an indicator of glycolytic capability, lactate dehydrogenase which is involved in the interconversion of lactate and pyruvate, and citrate synthase as an indicator of aerobic capacity of the tricarboxylic acid cycle. We found significant increases in lactate dehydrogenase and citrate synthase activities in both the right and left ventricles of high altitude fetal hearts, but no change in the activity of pyruvate kinase (Fig. 10). Thus, as judged by these enzyme activity changes, the high altitude heart displayed an enhanced ability to use its primary fuel, lactate^{9,10}, but no increased ability to metabolize glucose. To completely characterize the utilization of glucose and lactate in energy production, a number of other key enzymes in both the glycolytic and TCA pathways must be examined, as well as the function of the electron transport chain. If the high altitude fetal heart is capable of enhanced energy production, as suggested by the changes in lactate dehydrogenase and citrate synthase, these hearts should be able to sustain stronger contraction in hypoxic conditions compared to control hearts. To test this idea, we measured maximum developed tension (Tmax) in isolated papillary muscle subjected to varying levels of oxygen tension in the bath solution. When the bath was equilibrated with 20% oxygen (baseline is 95% oxygen), Tmax in control hearts was reduced to approximately 30 and 40% in the left and right ventricles, respectively. However, Tmax in the high altitude hearts was reduced to only approxi-

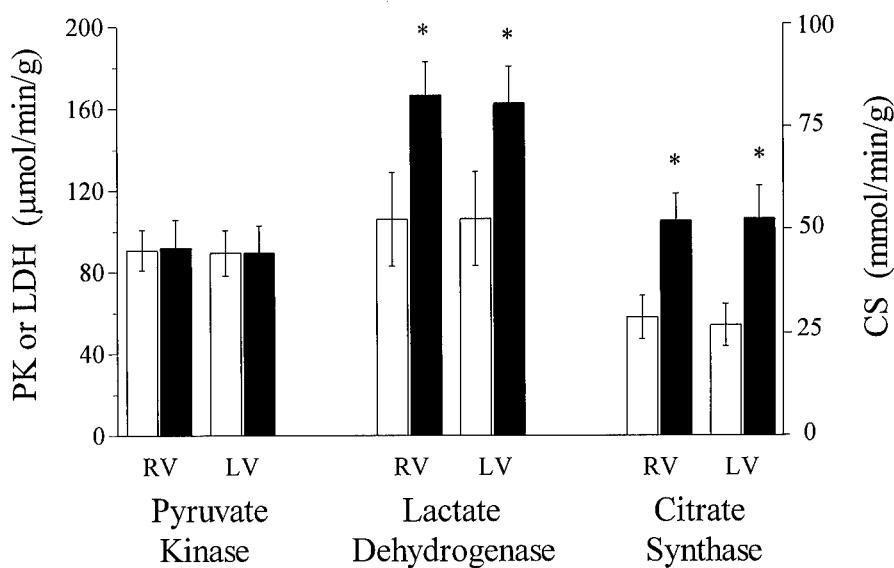


Figure 10 Activities of pyruvate kinase, lactate dehydrogenase, and citrate synthase in the left (LV) and right (RV) ventricles of control (open bars) and high altitude (black bars) fetuses. * significantly different from control, $p < 0.05$.

mately 45 and 55% in the left and right ventricles. These data are consistent with the hypothesis that the high altitude hearts are capable of greater energy production to sustain stronger contractions in hypoxic conditions.

Summary

In summary, the fetus responds to long-term (110 days) high altitude (3820m) with a decrease in total cardiac output, that is almost entirely due to a decrease in right ventricular output. It redistributes the decreased output to maintain blood flow to the heart and brain at normal levels at the expense of reduced flow to the body. In the myocardium of the high altitude fetus there was no change in the number of L-type calcium channels in either ventricle, but an increase was noted in SR calcium channels in the right ventricle. The right ventricle of high altitude fetuses also showed a greater dependence on calcium stored in the sarcoplasmic reticulum for initiation of contraction, as demonstrated by blocking of SR calcium release with ryanodine. β -adrenergic receptor enhancement of myocardial contractility was reduced in only the left ventricle of intact high altitude fetuses, but a reduction was demonstrated in isolated papillary muscle from both right and left ventricles. An increase in β -adrenergic receptor number was seen in only the right ventricle of high altitude fetuses. Energy production in high altitude fetal hearts appears to be enhanced as judged by increases in the metabolic enzymes lactate dehydrogenase and citrate synthase, as well as the ability of isolated papillary muscle to sustain stronger contractions in hypoxic conditions. Clarification of the intracellular mechanisms involved in the changes observed in high altitude fetal hearts will involve further studies of the calcium pathway re-

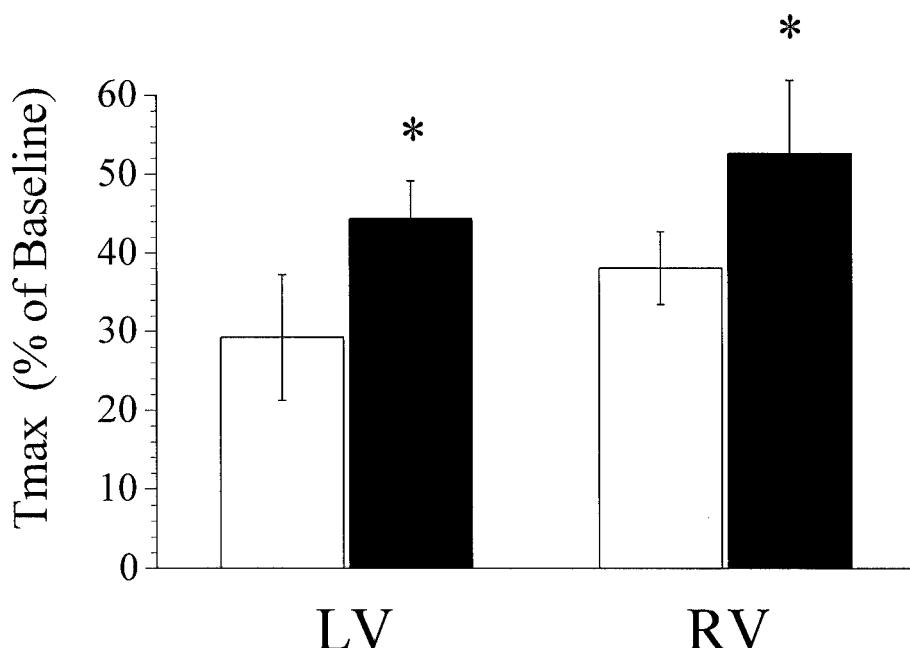


Figure 11 Reduction in maximum developed tension (Tmax) of isolated papillary in a bath system equilibrated with 20% oxygen (baseline is 95% oxygen) in the left (LV) and right (RV) ventricles of control (open bars) and high altitude (black bars) fetuses. * significantly different from control, $p < 0.05$.

sponsible for the initiation of contraction, the augmentation of contractility by the β -adrenergic receptor system, and of energy production.

Acknowledgements

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CHAPTER 13

THE NEWBORN AT HIGH ALTITUDE:

CARDIOPULMONARY FUNCTION

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Background

The transition which occurs at birth, in which the lungs change from fluid-filled to air-filled, pulmonary blood flow increases dramatically, and vascular shunts reverse in direction and close, is one of the most dramatic series of physiological events in the human lifespan. These events can occur successfully under the hypoxic conditions of high altitude, but the transitions are not without perils.

Soon after arrival of the Spaniards in the Andes in the mid-16th century, the new settlers expanded the ancient city of Potosi, a silver-mining center at an altitude of 4000m. The historian Antonio de la Calancha wrote that in the early years, no Spanish infants born in the city survived¹. Immigrant mothers began the practice of descending to nearby valleys to give birth. Their children remained at lower altitude until more than a year of age, when they would be brought up to Potosi. The first Spanish infant to survive after delivery in Potosi was born 53 years after the founding of the city, on Christmas Eve 1598.

While few details of the circumstances surrounding the birth and death of infants in the 16th century are available, it is easy to imagine neonates of low birthweight, maximally susceptible to cold stress, who achieved only very low arterial oxygen saturations without the availability of supplemental oxygen. Death could result from hypothermia, hypoglycemia, or dehydration, as well as apnea or extreme hypoxemia perhaps resulting from persistent pulmonary hypertension of the newborn.

With a focus on arterial oxygen saturation, ventilation, and the pulmonary circulation in the newborn, it is possible to identify altitude-related alterations in function, instances of successful adaptation, and examples of morbidity and mortality related to birth or residence at high altitude. Throughout the evidence two themes are interwoven: differential effects with increasing altitude and differences in response among various population groups.

Arterial Oxygen Saturation

Arterial oxygen saturation plays a critical role in postnatal transition because of its function as a pulmonary vasodilator. In addition to physical expansion of the lungs

with the first several breaths, alveolar oxygen dilates the pulmonary arterioles, allowing increased pulmonary blood flow after birth.

At sea level, SaO_2 immediately after birth is between 47 and 61% as measured by pulse oximetry. By 7 minutes SaO_2 has risen to more than 80%¹¹. SaO_2 remains somewhat lower in all infant activities during the first week of life ($91.2 \pm 3.7\%$ to $96.2 \pm 2.6\%$) as compared to the remainder of the neonatal period and infancy when SaO_2 values are between 94% and 98%¹⁸.

At an altitude of 1610m in Denver, Colorado, pulse oximetry performed in term, appropriate-weight-for-gestational age, healthy infants showed mean SaO_2 between 92 and 94% from 24-48 hours age through age 3 months while awake, sleeping, and feeding²⁶. At 24 to 48 hours of age, there was no effect of infant activity on saturation. At 1 and 3 months of age, SaO_2 was higher while awake and feeding than during sleep. Between 24-48 hours and 1 month of age, SaO_2 while awake increased slightly, but significantly, in a pattern similar to that seen at sea level.

At 3100m in Leadville, Colorado¹⁹ mean arterial oxygen saturation ranged from $80.6 \pm 5.3\%$ to $91.1 \pm 1.7\%$ from 6-24 hours age through 4 months (Fig. 1). SaO_2 fell during the first week after birth and then rose gradually to attain near-birth values at 2 and 4 months of age. At 6-24 hours and 24-48 hours, there was no effect of infant activity on saturation. At and after 1 week of age, values were higher during wakefulness than during active or quiet sleep. Feeding was intermediate between quiet awake and sleep.

The decrement in SaO_2 at 3100m compared to 1610m is similar to or slightly more than the reduction observed in adults. The pattern of higher SaO_2 while awake than during sleep is consistent with observations at 1610m and sea level; however, the fall in SaO_2 at 1 week differed from the pattern observed at lower altitudes. The drop in SaO_2 was consistent with clinical observations that babies who develop signs of hypoxemia (e.g. cyanosis, irritability, poor feeding, and failure to gain weight) often become symptomatic around 1 week of age.

At 3658m in Lhasa, Tibet, SaO_2 was examined by pulse oximetry in a group of 15 Tibetans and 15 Han neonates born in Lhasa²⁰. By study design, the Han and Tibetan mothers differed in altitude of birth and duration of high-altitude residence (Table 1). The neonates were similar in gestational age and Apgar scores; however the Han had lower birth weights and higher hemoglobin and hematocrit than the Tibetans. SaO_2 was higher in the Tibetans from birth through 4 months age. In both groups SaO_2 was highest in the first two days after birth (92 ± 3 and $91 \pm 4\%$ while awake at one and two days in the Han and 94 ± 2 and $93 \pm 2\%$ in the Tibetans) and fell by one week to $87 \pm 6\%$ in the Han and $89 \pm 3\%$ in the Tibetans (Fig. 2). SaO_2 during wakefulness was greater than during sleep at one week. SaO_2 in the Han declined progressively to $76 \pm 5\%$ during quiet sleep at 4 months, while the Tibetans' values stabilized at $86 \pm 5\%$.

Tibetans are unique in their length of ancestry at altitude, the lack of admixture with other populations, and the absence of migratory patterns to low altitude. The native Quechua population in Peru is analogous to the Tibetans with respect to long ancestry at high altitude. At an altitude of 3750m in La Oroya, Peru, SaO_2 values in 2- to 5-month-old infants averaged $87.8 \pm 2.6\%$ ²¹.

At the extreme altitude of 4540m in Morococha, Peru directly measured arterial saturations ranged from 57% to 75% in newborn infants from 30 minutes to 72 hours of age⁸. SaO_2 at this altitude remained in the range of 74-80% throughout infancy²⁴.

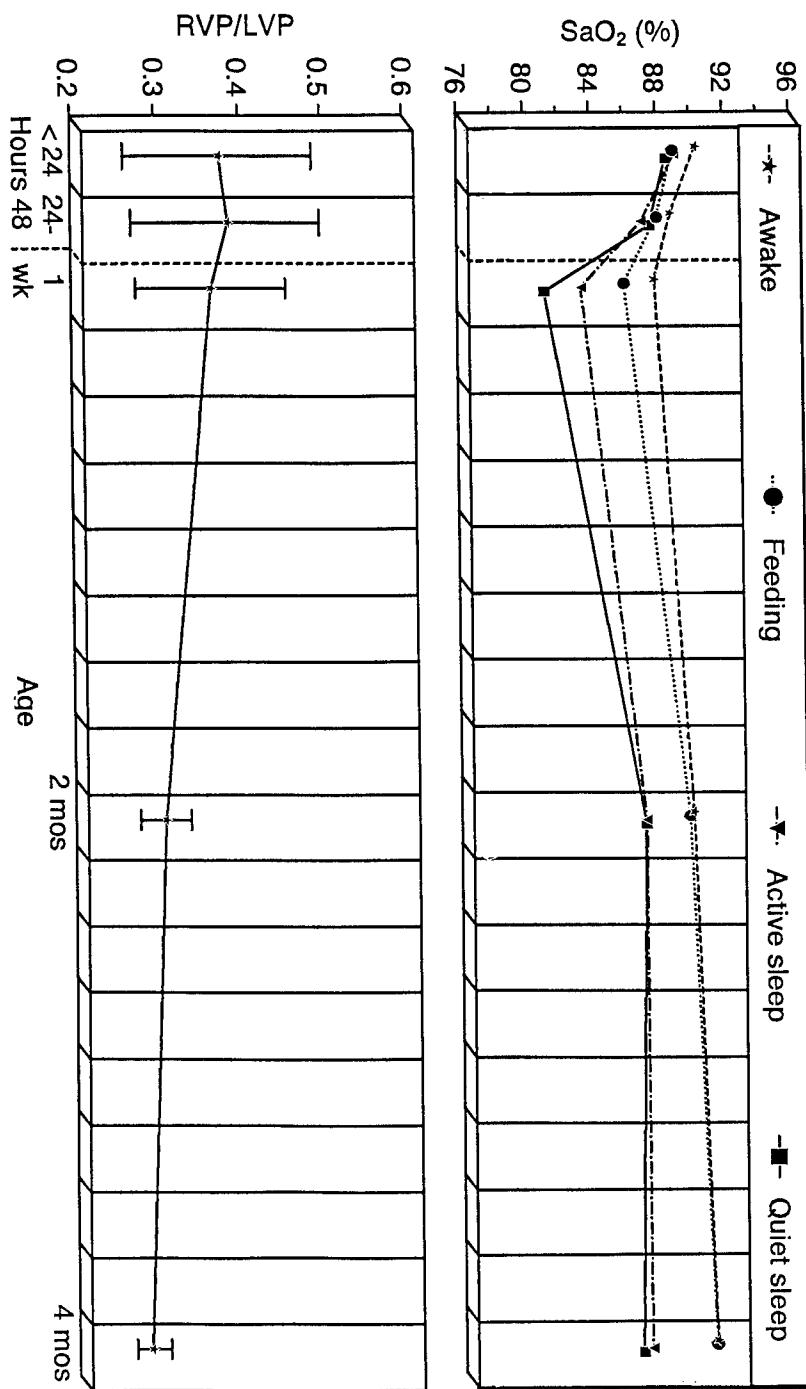


Figure 1 Arterial oxygen saturation (SaO_2 , percent) in infants at 3100m while awake, feeding, and during active and quiet sleep, and RVP/LVP ratio (mean \pm SEM) at each study period.

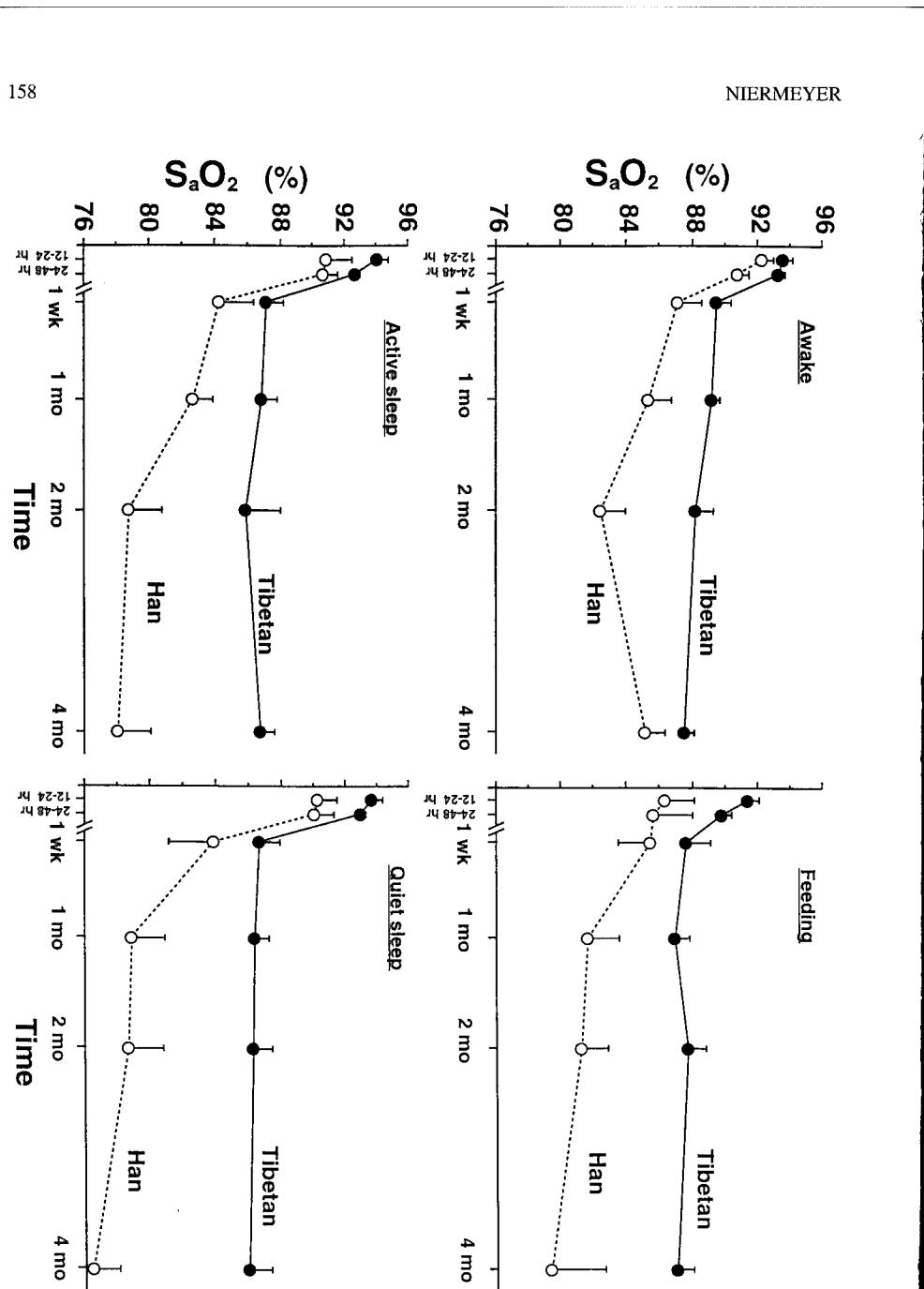


Figure 2 Arterial oxygen saturation (S_aO_2 , percent) in Tibetan and Han infants at each study time while awake, feeding, in active sleep and in quiet sleep (Mean \pm SEM). Tibetan values were greater than Han ($P < 0.05$) at 1 mo. and beyond in all conditions except waking and feeding at 4 mos.

Table 1. Group Characteristics of Han and Tibetan Mothers and Infants

VARIABLE	HAN	TIBETAN
Maternal		
Age, yrs	25±1	25±1
Parity	1.3±0.1	1.3±0.1
Altitude of birth, m	< 500	> 3000*
Residence > 3000m, yrs	2±1	25±1*
Infant		
Birth weight, g	2773±92	3067±107+
Gestational age, wk	38.9±0.4	38.9±0.4
Sex	8F, 7M	9F, 6M
Apgar score—1 minute	8	8
5 minute	10	9
Cord blood hemoglobin, g/dl	18.6±0.8	16.7±0.4+
Cord blood hematocrit, %	58.5±2.4	51.4±1.2#

Mean ± SEM.

*P < 0.001, +P = 0.04, #P = 0.01

To convert values for hemoglobin to grams per liter, multiply by 10.

Comparing the saturations obtained at 1610m, 3100m, and 3658m with similar study designs and the same oximeter, there is an evident decrease in saturation with increase in altitude. While oxygen saturation decreases as altitude increases, the change in saturation is not linear as hyperventilation occurs to varying degrees, leading to a smaller drop in alveolar oxygen. Hyperventilation also increases pH and thus shifts the oxyhemoglobin dissociation curve to the left²⁸. Differences in SaO₂ between wakefulness and sleep states are prominent at high altitude. There exist dramatic differences in SaO₂ between infants of two population groups residing in the same location but differing in their high-altitude ancestry.

Breathing Patterns

As hypoxia alters breathing patterns in adults, it has similar effect on respiration in neonates. Data on periodic breathing in neonates are highly variable due to different definitions of the event, various monitoring methods, and subjects across a range of postconceptual and postnatal ages. Periodic breathing is seen in up to 78% of full-term neonates in the first 2 weeks of life at sea level¹². Its prevalence declines with postnatal age—perhaps as early as 1 month and definitely by 5-6 months¹⁰. Exaggerated periodic breathing and apnea during hypoxia are well recognized in the preterm neonate^{1,22}.

In 1935 Deming and Washburn studied the rate, volume, and character of respiration in healthy infants from 20 hours to 13 weeks of age in the nursery of the Salvation Army Home in Denver⁶. Using a specially constructed respiratory chamber, spirometer, and kymograph, they found periodic breathing in the 27 infants studied to be “much more frequent and less transitory . . . than has been previously described.”

They described three principal types of breathing: regular, cog-wheel with quick inspiration and prolonged expiration, and periodic.

Sixty-five percent of newborn infants in Denver and 100% of neonates in Leadville showed irregular respiratory patterns in an early study by Lubchenco¹⁵. The infants with periodic breathing exhibited a repetitive pattern of 4 to 6 breaths over 6-7 seconds followed by a pause of 6-7 seconds.

Current investigations in Leadville are examining the relationship between breathing patterns and arterial oxygen saturation. At 24-48 hours, 1 week, 1 month and 3 months infants are studied using pulse oximetry and respiratory inductive plethysmography. Preliminary results confirm the occurrence of periodic breathing in all infants older than 48 hours. Periodic breathing is observed in both active and quiet sleep and is associated with a cyclic pattern of oxygen saturation; often the lowest saturation occurs after the onset of respirations. The duration of periodic breathing is prolonged as compared to that reported from sea level. This phenomenon may well provide partial explanation for the significant drop in mean saturations at one week of life and the persistence of lower saturations in sleep through 4 months at high altitude.

Periodic breathing may represent an exaggeration of normally occurring oscillations in respiratory frequency and tidal volume; thus it may constitute a phase of normal developmental changes in respiratory control^{23,27}. Several models have been proposed for feedback loops regulating periodic breathing; in these models loop gain is dependent on chemosensitivity, mixed venous pCO₂, circulatory delay, functional residual capacity, and cardiac output^{23,29}. Peripheral chemoreceptor reflexes and the interaction of central and peripheral chemoreceptors may be especially important in the regulation of periodic breathing in newborns¹⁰. The absence of periodic breathing in the first 48 hours of life is attributed to a functionally inactive carotid chemoreceptor during this period^{4,14}. Recent work in newborn animal models supports the idea of age-related changes in chemosensitivity during the first days of life¹⁶.

Pulmonary Circulation

The postnatal fall in pulmonary artery pressure (PPA) is central to achieving higher PaO₂ and effecting first functional, then anatomic closure of the atrial and ductal shunts. The strongest data demonstrating a prolonged postnatal fall in pulmonary artery pressure come from extreme high altitude.

Newborns at 4540m in Peru (P_B 446 mm Hg, alveolar oxygen partial pressure 50 mm Hg) showed persistence of near-systemic pulmonary artery pressure values for several days following birth⁸. In contrast, a rapid postnatal fall to adult levels of pulmonary artery pressure occurs in the first 3 days of life at sea level⁷. At high altitude right-to-left ductal shunt was documented until 5 hours; bidirectional flow occurred for another 5 hours; left-to-right flow was documented at 72 hours, when mean aortic pressure slightly exceeded mean pulmonary artery pressure. In 3 infants, administration of 100% oxygen at 72 hours resulted in a dramatic fall in PPA to values near those of infants at sea level⁸.

These findings are confirmed by right heart cardiac catheterization data from infants and children under 5 years of age at 4330m and 4540m in Peru who had elevated pulmonary artery pressures and increased pulmonary vascular resistance²⁴. Regression of the fetal pulmonary vascular pattern was delayed in South American children living at high altitudes³. Thickening of the muscular layer of small pulmonary arteries

and muscularization of the pulmonary arterioles regressed slowly; in some cases a fully adult pattern was never reached.

The prolonged course of decrease in pulmonary vascular resistance and pressure and right ventricular dominance is associated with delayed functional closure of the foramen ovale and ductus arteriosus. Gamboa, Marticorena, and Peñaloza reported patent ductus arteriosus in 0.74% of children in Cerro de Pasco at 4330m in contrast to 0.05% in the children of Lima at sea level⁹. An increased prevalence of atrial septal defect and patent ductus arteriosus was confirmed in a study of school children in three high-altitude sites in Qinghai Province, China. Prevalence increased from zero at sea level to more than 5% at 4500m¹⁷. Elevated right atrial pressure diminishes the pressure differential between right and left atria which causes the flap of the foramen ovale to close and functionally seal the atrial septum. Oxygen is a stimulus to ductal closure in the neonate; decreased oxygen tension is presumed to contribute to the increased prevalence of patent ductus arteriosus at high altitude^{2,9,17}.

In Lhasa at 3658m fifteen infants and children who died at ages 3 to 16 months with signs of pulmonary hypertension and right heart failure defined the syndrome of subacute infantile mountain sickness²⁵. Clinical signs and symptoms included dyspnea, cough, cyanosis, sleeplessness and irritability, facial edema, hepatomegaly, and oliguria. Histologic findings included medial hypertrophy of small pulmonary arteries, muscularization of pulmonary arterioles, and severe right ventricular hypertrophy and dilation. All children were Han except one Tibetan boy, and all but two children were born at low altitude and brought to Lhasa an average of 2 months prior to presentation. A control group of native Tibetan infants and children who died of non-cardiopulmonary causes showed normal thin-walled pulmonary arteries and arterioles after 4 months of age.

In Leadville (1610m) Khoury and Hawes reported 5 infants and 6 older children with a similar clinical syndrome¹³. Cardiac catheterization performed in 3 infants revealed pulmonary hypertension; one infant who died showed medial hypertrophy and intimal thickening of pulmonary arterioles and disruption of the internal elastic lamina.

In conjunction with recent studies of arterial oxygen saturation in Leadville, echocardiography was performed to assess indices of pulmonary artery pressure¹⁹. Using the left ventricular systolic circular index technique to obtain the ratio of right ventricular pressure to left ventricular pressure (RVP/LVP), values were within the normal to moderately elevated range during the first week of life and within the normal range in all 2- to 4- month infants. Of note is that all neonates in Leadville receive supplemental oxygen in the immediate postnatal period.

Current studies in Leadville are using Doppler echocardiography to document tricuspid regurgitation and estimate pulmonary artery pressure. Preliminary findings are consistent with those from the previous study.

At very high altitudes and without the use of supplemental oxygen, the postnatal fall in pulmonary artery pressure is quite slow. Fetal cardiovascular patterns persist into childhood in South American populations; whereas pathologic data in Tibetan infants indicate a more rapid involution of fetal patterns, again suggesting population differences in adaptation to high altitude. Even at lower altitudes, susceptible individuals may suffer from symptomatic pulmonary hypertension; however, persistent pulmonary hypertension does not appear to be prevalent at moderate altitude.

Conclusion

The newborn at high altitude experiences a slower transition from fetal to mature patterns of cardiopulmonary function. Arterial oxygen saturation rises slowly after birth and remains lower than at sea level. SaO_2 declines by one week of age and is lower in sleep than wakefulness. Certain populations show an exaggerated and prolonged fall in SaO_2 associated with clinical signs of hypoxemia. Periodic breathing patterns are observed in all neonates at high altitude. The duration of periodic breathing in sleep is greater than at sea level and is associated with cyclic changes in SaO_2 . Pulmonary artery pressure falls very slowly with persistence of fetal pulmonary vascular patterns and a higher prevalence of persistent right-to-left shunts. Symptomatic pulmonary hypertension may develop in susceptible infants at high altitude.

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CHAPTER 14

MONGE'S DISEASE: 70 YEARS AFTER ITS FIRST DESCRIPTION

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The Peruvian Andes have been the site of intensive research on the anthropology, physiology and pathology of human populations living permanently at high altitude.

The pre-Inca and Inca populations were known for their intensive migratory habits and their colonization of coastal communities. After the Spanish conquest, the Peruvian population was reduced to one tenth its original number. Thus, the genetic pool was diluted, to be slowly recomposed with an admixture of races.

These factors acted against the natural selection, in the Darwinian sense, of those most fit for life at high altitude, thus resulting in a weak genetic selection. On the other hand, humans have a sea-level physiological design which requires a strong natural selection for the hypoxic environment. This negative combination must be taken into account when considering the problem of chronic mountain sickness.

Chronic mountain sickness or Monge's disease has a biological basis. It should be considered as a disease of the population and not as a discrete clinical entity affecting only a few individuals. I will present a brief summary of some important Peruvian investigations carried out on this subject. To shorten the presentation I will use illustrations when appropriate.

In 1925 Carlos Monge-M (Monge Medrano in Peru), reported to the National Academy of Lima his first case of chronic mountain sickness and titled it "Váquez's disease (high altitude erythremic syndrome)". At that time, this name was used to describe cases of pathological polycythemia¹⁴. (Fig. 1).

In 1927 he organized an expedition to Cerro de Pasco (Peruvian Andes, 4300m) in order to refute Joseph Barcroft's affirmation that acclimatization to high altitude did not exist. The results were published in 1928. (Fig. 2)^{15,6,17}. Although the book's intention was to refute Barcroft and demonstrate that the high-altitude native was fully acclimatized, it was titled "The Disease of the Andes" and contained the first detailed description of chronic mountain sickness. It seems that Monge-M's need to show the medical importance of this new clinical entity predominated over his interest in refuting Barcroft.

The name Monge's disease was first proposed by Professor H. Váquez (Marcos Cueto, personal communication) to Professor G.H. Roger, Dean of the School of Medicine in Paris, who suggested the use of this name in the preface of a book in French, written in 1929¹⁶. During an expedition to Chile in 1935, J. Talbott and D.B. Dill found a case of chronic mountain sickness and independently proposed the same name for this clinical entity.¹⁹ (Fig. 3).

1925. Carlos Monge M. Lima

Sobre un caso de Enfermedad de Váquez (Síndrome eritrémico de altura). Presented to the National Academy of Medicine of Lima in 1925, the same year Barcroft published "The Respiratory Function of the Blood: Lessons from High Altitude"

Carlos Monge M. 1927 expedition to Cerro de Pasco

"La Enfermedad de los Andes" (1928). Andean natives are fully acclimatized. Chronic Mountain Sickness is the loss of acclimatization. Why then the book's title "The Disease of the Andes? . . . (Monge-C has an answer).

Figure 1

Monge-M was influenced by the writings of Father F. de Acosta in the XVI century and F. Viault in the XIX century. During the entrance examination to the San Marcos University School of Medicine, Viault was able to answer correctly a question on high altitude polycythemia. (Fig. 4).^{1,20,21} In his later years, he devoted himself to the cultural anthropology of high altitude populations intending to convince the Peruvian government on the need to improve the Andeans' way of life.

Alberto Hurtado played a fundamental role in the study of high altitude acclimatization in the Peruvian Andes. Because the University of San Marcos was closed due to political disturbances, he went to Harvard University where he completed his studies in its prestigious medical school. On his return to Peru, he joined Monge-M during the 1927 expedition.

Hurtado studied excessive arterial blood oxygen unsaturation and excessive polycythemia in chronic mountain sickness. As its cause, he suggested primary hypoventilation. Together with his associates, he described an elevated pulmonary arterial pressure in high altitude natives. Figure 5 shows some of Hurtado's contributions to high altitude physiology and medicine. He wrote a classical article on human physiology at high altitude in the American Handbook of Physiology⁷.

J. Arias-Stella and M. Saldaña made a classical description of the bronchopulmonary tree pathologic anatomy of the high-altitude natives³. Arias-Stella found an enlargement of the carotid bodies in high altitude natives and described their histol-

1921-22. J. Barcroft Peruvian Andes

“And this redistribution of disadvantages appears to be the essence of acclimatization. The acclimatized man is not . . . who has attained to bodily and mental powers as great in Cerro . . . as he would have in Cambridge. . . All dwellers at high altitude are persons of impaired physical and mental powers . . .”

Carlos Monge M. Comments on Barcroft’s opinion

1948. “Professor Barcroft was himself suffering a subacute case of mountain sickness. His substantial error . . . resulting from an improper generalization on his part of what he himself felt . . . to Andean man in general . . .”

Figure 2

Carlos Monge M. Monge’s Disease

Dean G.H. Roger suggested the name: “la maladie de Carlos Monge”. Preface of “Les Erythrémies de L’Altitude”, 1929.

J. Talbott and D.B. Dill reported one case of chronic mountain sickness found during the 1935 expedition to Chile. Am J Med Sci, 1936. Proposed the name “Monge’s Disease”.

Figure 3

ogy^{2,4}. Saldaña¹⁸ found that the incidence of chemodectomas was 10 times higher in high altitude natives than in sea level residents. (Fig. 6).

In a longitudinal study, J. Whittembury and C. Monge-C (Monge Cassinelli in Peru) found that high altitude natives living at 4500m markedly increased their hema-

1573. Father Acosta Peruvian Cordillera

“I am persuaded that the element of air is so thin and delicate that it does not provide for human respiration which needs it to be thicker and more tempered”.

1889. F. Viault. Peruvian Cordillera

“One may suppose *a priori* that the physiological reason that allows man and animals to endure the very rarefied atmosphere of high places must be the result of either:

an increase in the frequency of respiratory movements;

or an increase in ... the red blood cells;

or greater respiratory capacity of the hemoglobin

- a reduction in the oxygen needs of the tissues;
- a reduction in the amount of tissue oxidation,
- a higher work efficiency for the oxidation that has occurred”.

Figure 4

tocrit with age. They suggested that Monge's disease might be the result of a basic lack of acclimatization, even though the subjects were born and living at high altitude. Sime *et al* (1975) described a close correlation between the decrease in ventilation and the increase in hematocrit with age. Monge-C and Whittembury¹² predicted the rise in hematocrit with age at high altitude by means of a simple mathematical model.

Fabiola León-Velarde and Alberto Arregui have carried out an intensive epidemiological study in Cerro de Pasco (75,000 inhabitants) at 4300m. It confirms our

**Alberto Hurtado
MD from Harvard University**

Returns to Peru and joins Monge-M. during 1927 expedition.
Chronic mountain sickness: describes excessive blood unsaturation and polycythemia. Suggests primary hypoventilation as the cause. Classical paper in Handbook of Physiology, 1964.
HA elevated arterial pulmonary pressure (1956)

**Alberto Hurtado
Morococha Laboratory (4540 m)**

Considers maximal exercise capacity of native as the same or superior to SL men. Confirms hypolactacidemia. Finds P_{50} slightly deviated to the right. Increased muscle myoglobin in HA dogs. Links Peruvian research to Harvard Fatigue Laboratory and to University of Rochester, NY.

Figure 5

**J. Arias-Stella and M. Saldaña
Pathologists**

Classical description of the pathologic anatomy of the bronchopulmonary tree in high-altitude natives (1963).
Arias-Stella finds enlargement of carotid bodies in HA natives and describes histology (1969).
Saldaña et al: incidence of chemodectomas 10 times higher in HA natives.

Figure 6

studies that, as age increases, polycythemia becomes excessive and the symptoms and signs of chronic mountain sickness also increase^{13,5,9}.

CMS ANDEANS

- Excessive hypoventilation
- Excessive polycythemia
- Excessive pulmonary hypertension
- Excessive chemoreceptor function?
(hyperplasia)
- Aging and increasing polycythemia in man

Figure 7

Our present research team at the Universidad Peruana Cayetano Heredia believes that mammals have a sea-level physiological design because the hemoglobin saturates with oxygen at the sea-level value of 100 torr. Any elevation above sea level will diminish this value and stimulate erythrocytosis, which can be maladaptive²³. Most probably, during evolution, the erythrocytic response was developed in order to correct anemic hypoxia and not hypobaric hypoxia. In the hypoxic environment erythrocytosis is stimulated above the normal requirements of O₂ concentration in the blood.

At high altitude, the normal decay of the ventilatory rate with age will induce excessive polycythemia, excessive pulmonary hypertension and possibly excessive function of the chemoreceptors that may end in chemodectomas. Comparative physiology studies show that these changes do not occur in genetically adapted animals but that they happen in men and in mammals introduced in South America during the Spanish conquest. (Fig. 7).^{23,11}.

Monge's disease is a multifactorial pathological condition¹⁰ that seems to have a biological basis in the human population. Young people can tolerate life at high altitude and reproduce as well as any comparative sea level population. As age increases, excessive polycythemia follows and, as a result, the symptoms of chronic mountain sickness appear and impede a normal life even in people who have lived in the mountains for generations. Fortunately, the condition disappears at sea level, and it is improved by bloodletting.

Monge-M became prophetic at the end of his presentation of the first case of chronic mountain sickness in 1925. He said that his intention in presenting a new medical entity was not to show his clinical skills but to call the attention of the Peruvian health authorities to a disease that he considered important for the welfare of mountain populations. It took 70 years of integrative research to find that his prophecy could be proved experimentally, clinically and epidemiologically.

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CHAPTER 15

NITRIC OXIDE: MECHANISM OF ACTION AND ROLE IN HUMAN PATHOPHYSIOLOGY

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Introduction

Nitric oxide (NO) is an unstable radical which acts as a messenger molecule to modulate a variety of biological processes (see (47,81) for reviews). As will be discussed in this chapter, NO serves as a mediator of neurotransmission, regulates vascular tone and functions as an immune effector and regulator. In fact, a role for NO has been postulated in almost every mammalian organ system. NO is synthesized in a variety of cells including endothelial cells, vascular smooth muscle cells, neurons, hepatocytes, platelets, neutrophils, macrophages, islets of Langerhans and osteoblasts. The diversity of tissue distribution of NO synthesis is summarized in Figure 1. Dysfunction of the NO pathway may occur in pathophysiological states such as essential hypertension, pulmonary hypertension, sepsis and insulin dependent diabetes mellitus. This chapter will discuss the role of NO in humans and summarize recent advances in our knowledge of the mechanism of NO action.

The history of EDRF (Endothelium derived relaxing factor)

It was known for many years that man produces more nitrate than he ingests³⁵, suggesting the presence of a nitrogen oxide synthetic pathway. It was also known that nitroglycerin and related nitrosocompounds are potent vasodilators^{36,51,52}. The discovery of NO as an endogenous vasodilator explains how these seemingly unrelated observations are intertwined. Dating back to the 1800s, organic nitrogen oxide-containing compounds were used in the treatment of angina. In fact, Alfred Nobel, who invented dynamite in 1864 using a stable nitroglycerin preparation, ironically required the same compound himself for treatment of angina (albeit in a somewhat less explosive preparation)⁴⁷. NO and nitrovasodilators cause smooth muscle relaxation via activation of soluble guanylate cyclase with subsequent production of 3', 5' cyclic guanosine monophosphate (cGMP)^{36,50,52,83}. It was not until the discovery by Furchtgott and Zawadzki of a dilator substance produced by endothelium, that the quest to link NO and EDRF began³². Furchtgott and Zawadzki demonstrated that acetylcholine elicited vasodilation in excised strips of rabbit aorta only when intact endothelium

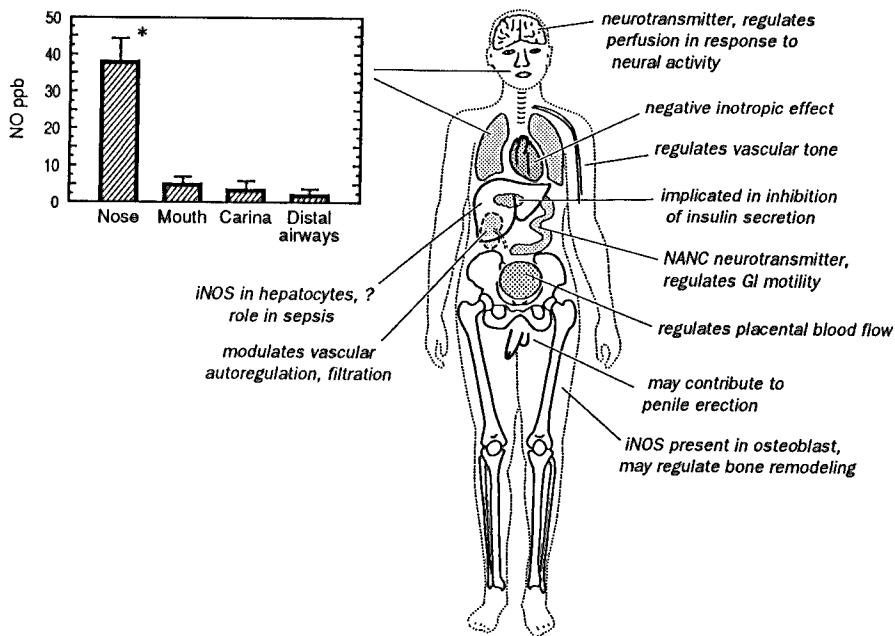


Figure 1 Tissue distribution of NO synthesis in man. Note the diverse functions of NO and its presence in virtually all organs. The insert graph depicts NO measured in parts per billion (ppb) from the nose, mouth, carina and distal airways. The predominant source of NO in the breath of man is the nose.

was present. Furthermore, the effluent from endothelium intact strips stimulated with acetylcholine was capable of dilating endothelium-denuded strips. The identity of this short-lived dilator substance was not clear. The identification of EDRF as NO proceeded in parallel with the realization that the cytostatic substance produced by activated macrophages was NO^{40,74,113}.

How do we know EDRF is Nitric Oxide?

Subsequent to the work by Furchtgott and Zawadzki, numerous studies presented strong evidence that EDRF is indeed NO, and as such, the term endothelium-derived nitric oxide, EDNO, has been promoted. The evidence for NO being the EDRF includes similarity between biologic EDRF and authentic NO in terms of their effects on vascular tone in bioassay, similar biologic half lives (5-30 seconds)^{48,49,88,89}, avid binding to iron-sulfur complexes, and their ability to increase cGMP in reporter cells⁸⁸. Furthermore, measurement of nitrogen oxides and later, of NO itself, confirmed the identity of NO as EDRF^{5,88}.

Although numerous nitrogen oxide containing compounds were tested as candidate EDRF substances, all differed from NO either in their stability, susceptibility to inactivation by oxyhemoglobin or in their reaction with cysteine²⁹. NO remains as the only substance identified so far with identical chemical, biochemical and pharmacological features to EDRF. It remains possible that EDRF is an unidentified nitrosocompound which spontaneously decomposes to NO, although EDRF does appear to be NO²⁹. Likely candidates include nitrothiol compounds. These compounds, produced when NO interacts with sulfhydryl groups in molecules such as

glutathione, can release NO spontaneously or in response to photostimulation. The biologic relevance of nitrosothiols as "NO transport" or "storage" molecules is uncertain²⁹. Most investigators agree that NO itself is the final molecule that stimulates guanylate cyclase.

NO synthases

NO is generated by oxidation of the terminal guanidino nitrogen atom of L-arginine by a family of dioxygenase enzymes, the nitric oxide synthases (NOS)⁸⁹. NOS combines L-arginine and molecular oxygen to form NO and L-citrulline. NOS are a family of heme-containing, NADPH-flavoproteins whose NADPH-flavoprotein sequences (the reductase portion of the enzyme) are remarkably homologous to cytochrome P-450 reductases (Fig. 2)¹¹². The oxidation of L-arginine begins as NADPH donates an electron to the flavin center¹¹¹. Electron flow from the reductase to the oxidase (heme iron) portion of the enzyme is controlled by calmodulin, in much the same way as an "electric switch" controls the flow of electricity⁷⁵. The stereospecific binding of L-arginine to the heme iron facilitates electron transfer to the heme center⁷⁵. The electron is then donated, together with an electron from molecular oxygen, to the terminal guanidino group of L-arginine to form the intermediate². A second molecule of oxygen and 0.5 NADPH donate electrons to this intermediate to form one molecule of NO and L-citrulline. Tetrahydrobiopterin (BH₄) is an important co-

NOS-a dimeric dioxygenase

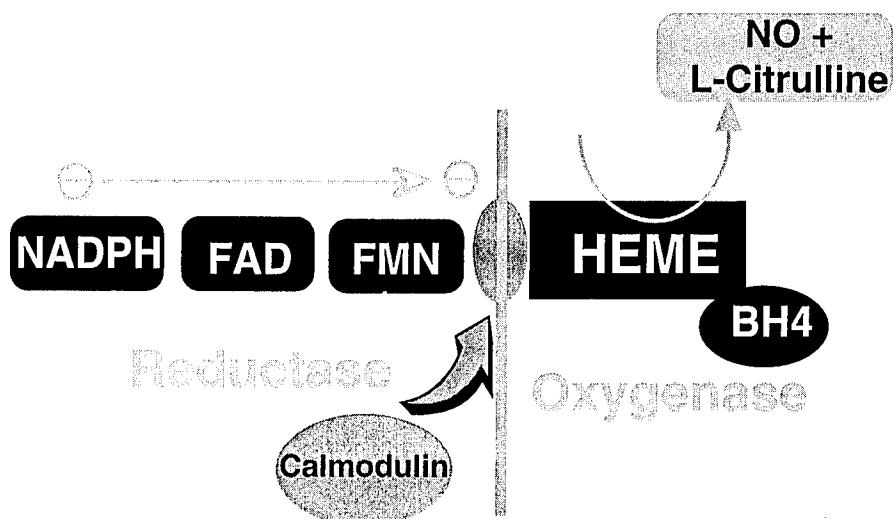


Figure 2 Nitric oxide synthases (NOS) are a family of heme-containing, NADPH-flavoproteins responsible for the conversion of L-arginine and molecular oxygen to NO and L-citrulline. The enzyme is comprised of a reductase and an oxidase portion. Calmodulin controls the flow of electrons from the flavin to the heme center. The role of tetrahydrobiopterin (BH₄) is unclear. The enzyme, which exists as a dimer, is depicted here as a monomer.

factor required for the catalytic function of NOS, although its exact role is unclear. Some evidence suggests that BH_4 stabilizes the dimeric structure of NOS¹¹.

While all NOS share the above characteristics, significant structural, functional and genomic differences exist, allowing for classification of at least three isoforms: neuronal (nNOS), endothelial (ecNOS) and inducible (iNOS) (see (61,84) for review). Each isoform has been sequenced, cloned and its chromosome location identified (Table 1). The structure of each isoform is highly conserved among mammalian species (>90%), although there is little homology between isoforms (<50%) within a single species (personal communication, Timothy Biliar and⁶⁰). nNOS and ecNOS are constitutively expressed, meaning the enzyme is present under normal conditions. Rapid NO synthesis (i.e., within seconds) occurs in response to an increase in cytosolic calcium and binding of calmodulin to the enzyme^{61,84,124}. In contrast, iNOS must be transcribed and translated in response to stimuli such as cytokines. Upregulation of NOS translation follows several hours after stimulation with cytokines or endotoxin (lipopolysaccharide, LPS)¹⁰⁸. It was originally believed that iNOS differed from the constitutive isoforms by the absence of calmodulin dependence. However, it now appears that calmodulin is tightly bound to the inducible enzyme. In contrast, calcium-calmodulin binds reversibly to the constitutive isoforms and these isoforms can be inhibited by inhibitors of calcium-calmodulin⁶¹.

Table 1

	ecNOS Type 3	iNOS Type 2	nNOS Type 1
Ca ²⁺ Sensitive	Yes	No	Yes
Inhibited by steroids	No	Yes	No
Dominant Source	Endothelium	Macrophage	brain
Chromosome	7	17	12
Gene Size	>20 kB	>20 kB	80-100 kB
Promoter has TATA Box	no	yes	?

The time course of NO production following a stimulus differs dramatically between constitutive and inducible NOS. NO production by the constitutive NOS isoforms increases rapidly in response to an appropriate stimulus. For example, NO synthesis in pulmonary artery rings and cultured pulmonary artery endothelial cells increases within seconds of administration of bradykinin or the calcium ionophore, A23187⁸. On the other hand, inducible NO production requires synthesis of new iNOS

mRNA and protein and, thus, NO production is delayed compared to cells expressing constitutive isoforms. As demonstrated in Figure 3, NO production by renal mesangial cells increases six hours after incubation with LPS. The time course of NO production follows the production of iNOS mRNA¹⁰⁸. Finally, iNOS differs from nNOS and ecNOS in that glucocorticoids suppress iNOS activity^{61,108}, perhaps by altering iNOS gene expression.

Constitutive NOS isoforms have been demonstrated in brain, endothelium, adrenal gland and platelets. iNOS expression has been demonstrated in hepatocytes, macrophages, neutrophils, osteoblasts, mesangial cells and bone marrow cells⁶¹. The structural differences between NOS isoforms offers the potential to develop isoform-specific inhibitors, which may aid in the treatment of disease states characterized by excess NO production.

Mechanisms for NO-induced relaxation

Nitric oxide, cGMP and the pulmonary vasculature

NO and nitrovasodilators cause pulmonary artery (PA) vasodilatation by activating guanylate cyclase and increasing cGMP levels in vascular smooth muscle (VSM)^{50,82,83}. Increases in cGMP precede relaxation, and inhibition of guanylate cyclase blocks both cGMP synthesis and relaxation, indicating a central role for cGMP in NO and nitrovasodilator-induced relaxation⁵¹. Drugs which increase cGMP levels may cause relaxation by one of four mechanisms, working either alone or in combination: activation of K_{Ca} channels^{9,104,115}, inhibition of the Ca channel¹²³, desensitization of the contractile apparatus to Ca and/or Ca sequestration by the sarcoplasmic reticulum (SR) (Fig. 4)¹²⁰. Although this chapter focuses primarily on the effects of cGMP on the K and Ca channels, Ca sequestration and diminished Ca sensitivity of the contractile proteins may occur by similar mechanisms and proceed simultaneously with the effects on ion channels.

Cyclic nucleotides alter the activity of ion channels by one of two mechanisms. Either they directly interact with the channel protein, as in the cation channels of the retinal rod cells⁷⁹, or their effects are indirect, through activation of cyclic nucleotide dependent protein kinases. These kinases control ion channel function by phosphorylating the channel or its regulatory factors. cGMP-dependent protein kinase (cGMP-PK) is a serine/threonine kinase, abundant in VSM, which is activated by low levels of cGMP (<1 μ M)^{26,71}. cGMP-PK phosphorylates a specific site on ion channels and other intracellular proteins (e.g. arg-arg-X-serine)^{26,71}. The major mechanism of NO and cGMP-induced relaxation examined in this chapter, activation of calcium sensitive potassium (K_{Ca}) channels in response to cGMP-PK, has recently been described in colonic cells¹¹⁷, as well as carotid¹⁰⁴ and pulmonary⁹ VSM. The independent reports in diverse types of smooth muscle suggest this mechanism is widely conserved and therefore is likely to be important.

K Channels and regulation of membrane potential in PA VSM

Membrane potential in VSM is largely controlled by K channels. When K channels open, positively charged K ions exit, leaving negatively charged macromolecules trapped in cell interior. This makes the interior of the cell more negative, relative to the exterior: "membrane hyperpolarization". Hyperpolarization inactivates the voltage-gated Ca channel, reducing Ca influx and inhibiting vasoconstriction. Thus, K

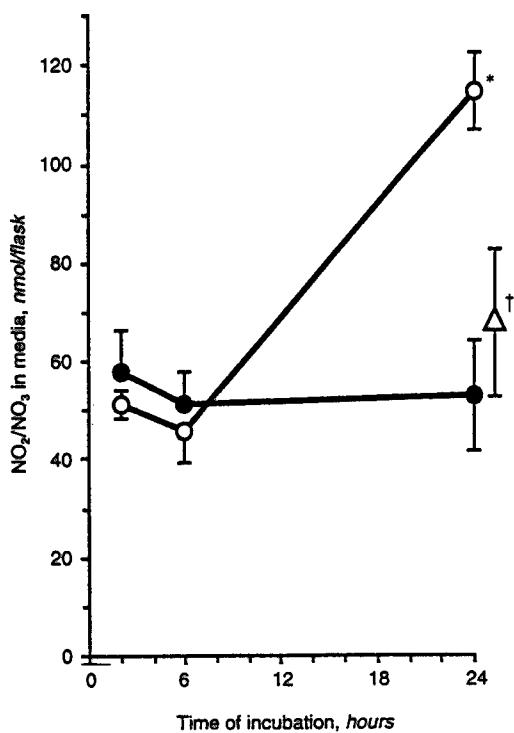
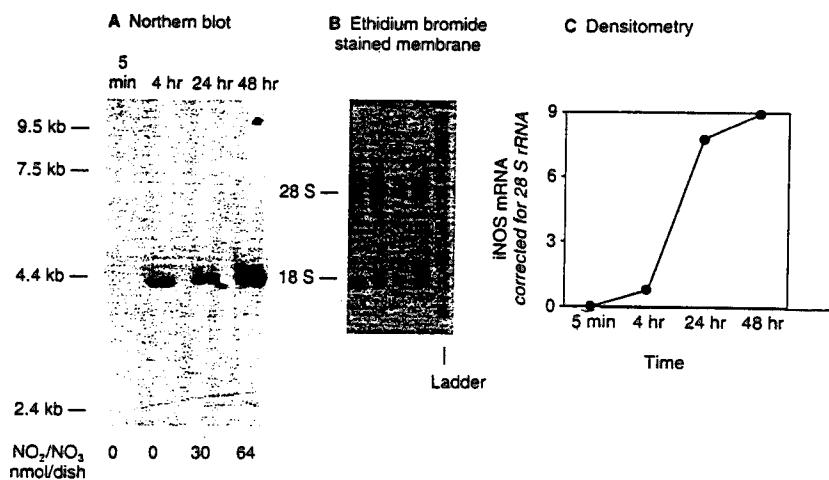


Figure 3 Time course of iNOS mRNA synthesis and NO production by renal mesangial cells incubated with endotoxin (LPS). **A.** Northern blot demonstrating appearance of iNOS mRNA (4.4 kb), beginning 4 hours following LPS stimulation. **B.** Ethidium bromide staining demonstrates similar total RNA in each column. **C.** Quantification of iNOS mRNA using densitometry. **D.** Nitrite and nitrate ($\text{NO}_2^-/\text{NO}_3^-$) measured in the media bathing renal mesangial cells as a function of time under control conditions (open circles) and following NOS inhibition with L-NAME (closed circles). Note the increase in $\text{NO}_2^-/\text{NO}_3^-$ 24 hours following LPS stimulation. This figure is reproduced with permission from reference 109.

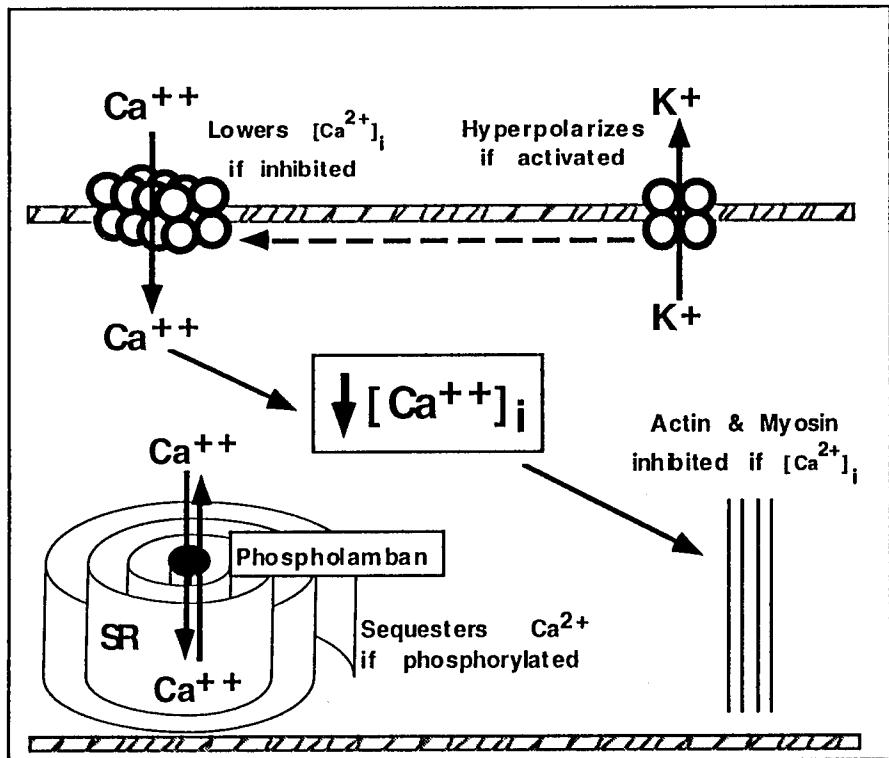


Figure 4 Mechanisms by which increased cGMP levels may produce relaxation of vascular smooth muscle: activation of K_{Ca} channels(9,104,115), inhibition of the Ca channel(23), desensitization of the contractile apparatus to Ca and/or Ca sequestration by the sarcoplasmic reticulum (SR).

channel blockers cause pulmonary vasoconstriction^{39,99} and K channel activators cause vasodilatation²⁰ largely through their effects on the membrane potential and the Ca channel. Although it has long been suspected that K conductance was important to the regulation of pulmonary vascular tone^{6,45,72}, the application of patch clamp techniques has provided direct evidence of the involvement of K channels in regulation of the pulmonary circulation. K channels in PA VSM are inhibited by hypoxia, leading to depolarization and pulmonary vasoconstriction^{100,125}.

PA VSM cells contain various types of K channels including: K_{Ca}⁹⁹, K_{DR} and adenosine triphosphate(K_{ATP})-gated K channels^{22,123}. K_{ATP} channels, though inactive in normoxia³⁹; do contribute to anoxic PA relaxation¹²³. Definitive identification of the K_{Ca} channel depends on use of single channel, inside out patches and measurement of open probability (P_o) at varying Ca concentrations and calculation of channel conductance in symmetrical K. However, the presence of this large conductance channel can be inferred in whole cell studies by the presence of spontaneous spiking on the current traces (due to opening and closing of large conductance channels) and their inhibition by charybdotoxin (CTX) or low doses (<10 mM) of tetraethylammonium (TEA)⁶⁷. The large conductance of K_{Ca} channels has importance in PA VSM cells which have high input resistance (few channels/mm² of membrane), since opening

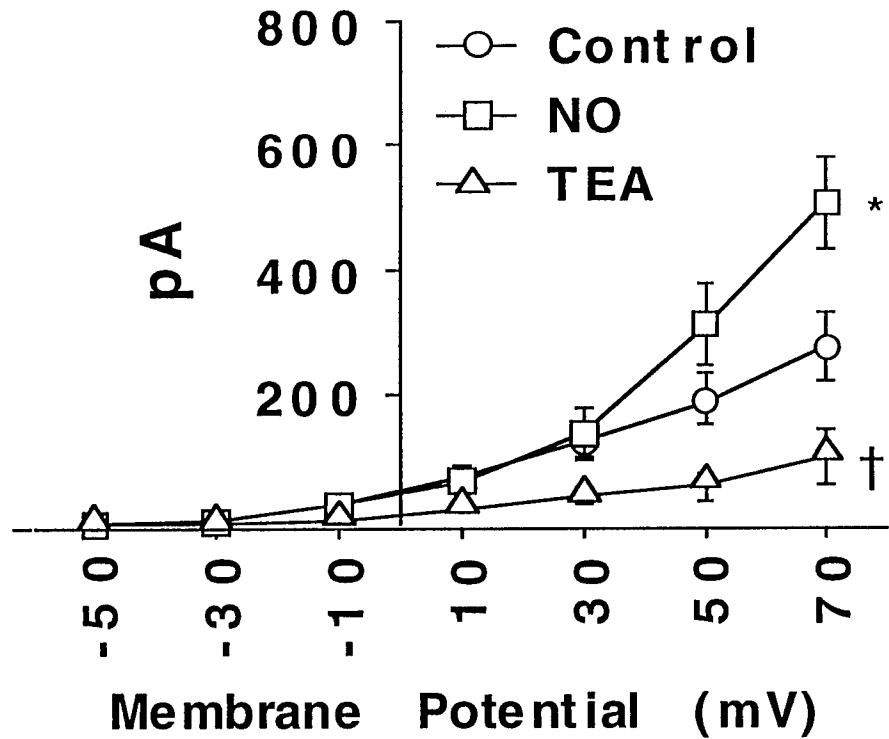
even a few channels could hyperpolarize the membrane. CTX³³ and iberiotoxin are preferential blockers of the high-conductance, K_{Ca} channels whereas small-conductance K_{Ca} channels are blocked by apamin³³. The small conductance K_{Ca} channels are present in PA VSM but their function is undetermined⁹⁹. K_{DR} channels, originally described in neural tissue, were so named because they displayed a voltage-activation delay. However, this property is now known to be common to many types of channel⁴³. K_{DR} channels are insensitive to glyburide and CTX and are preferentially inhibited by low dose 4-aminopyridine (4-AP, 1-5 mM)⁴³. TEA can inhibit both K_{Ca} and K_{DR} channels but at doses <10 mM, preferentially inhibits K_{Ca} channels. In our prior studies NO and cGMP activate K_{Ca} , but not K_{DR} channels⁹.

In order to understand the techniques for assessing the intracellular response to cGMP it is necessary to review the strategies we use for modulating cGMP levels, the activity of guanylate cyclase and cGMP-PK. cGMP itself is poorly absorbed by cells. Using conventional whole cell patch clamp technique, cGMP can be administered intracellularly as a constituent of the pipette solution. For perforated patch studies cGMP levels are increased by administering membrane permeable cGMP analogs (e.g. 8-bromo cyclic guanosine monophosphate, 8-Br-cGMP), activators of guanylate cyclase (NO) or inhibitors of cGMP phosphodiesterase (zaprinast). Soluble guanylate cyclase is activated by EDRF/NO and inhibited by methylene blue and LY83583. Methylene blue and LY83583 are both thought to interfere with cGMP synthesis by stimulating production of reactive oxygen species⁶⁴. LY83583 lowers lung cGMP levels¹⁰⁷.

Monophosphorothioate analogs of the cyclic nucleotides are highly selective agonists and antagonists for their respective kinases¹⁰⁵. cGMP-PK can be activated by (Sp)-cGMPS and inhibited by (Rp)-cGMPS, diastereomers of guanosine 3',5'-monophosphorothioate. (Sp)-diastereomers, have an axial exocyclic sulfur atom, bound to the cGMP-PK and stimulate phosphorylation⁶⁸. In contrast, (Rp)-cGMPS, has an equatorial exocyclic sulfur atom and antagonizes cGMP-PK (K_i of 20 mM). Cell permeable monophosphorothioate derivatives which activate and inhibit cGMP-PK (e.g. 8-pCPT-cGMPS & 8-pCPT-cGMP, respectively) permit the extracellular administration of these agents in perforated patch studies using intact cells⁶⁸. Other kinase inhibitors which preferentially target cGMP-PK, rather than protein kinase C or A, are also cell permeable (e.g. KT58323)⁴².

Effects of KCl and K channel Blockers on the NO/cGMP Pathway

There is substantial evidence that membrane potential is an important determinant of the ability of vessels to elaborate or respond to EDRF/NO. KCl, which depolarizes the membrane by decreasing the gradient for K efflux, inhibits EDRF activity in bioassay³¹ and impairs bradykinin-evoked elevations of $[Ca^{2+}]_i$ in endothelial cells^{3,55}. Even when authentic NO is given to denuded PA rings, KCl inhibits relaxation²⁶. This suggests that depolarization is preventing an important effect of NO on membrane potential rather than inhibiting NO synthesis. There has been controversy whether NO does^{34,66,116} or does not^{14,63} cause membrane hyperpolarization. The evidence supporting EDRF and NO as hyperpolarizing agents and K channel activators is, however, more complete and persuasive, including studies of intact vessels^{14,21,56,63,66,116} and patch-clamp documentation of the ability of cGMP and NO to activate K channels^{9,58,104,115} (Fig. 5). An example of NO hyperpolarizing PA VSM by opening K_{Ca} channels is provided, illustrating ongoing work in our laboratory (Fig. 6).



KCa CELL TYPE (n=5)

Figure 5 NO activates K_{Ca} channels in pulmonary vascular smooth muscle

This is a current voltage plot of amphotericin-perforated patch clamp data from 5 PA VSM cells (mean \pm SEM). NO increased the outward K current ($^* p < 0.05$ vs control) and TEA inhibited the NO response ($^† p < 0.05$ value differs from NO), suggesting the class of channels activated was the K_{Ca} channel.

NO/cGMP and the Ca channel

The pulmonary vasculature contains L-type Ca channels which are inhibited by dihydropyridine type blockers, such as nifedipine and nisoldipine^{7,76}. The contribution of Ca channels to pulmonary vasoconstriction is well established. Ca channel inhibitors decrease^{7,76} and activators (e.g. BAYK8644) enhance^{77,119} pulmonary vasoconstriction. However, the role of the Ca channel in NO-induced relaxation is uncertain. Supporting the concept that NO might act as a "Ca channel blocker", nitroprusside inhibits the Ca channel²³. The relative importance of a direct inhibitory effect of NO or cGMP on the Ca channel vs. a passive, voltage-dependent inhibition of the Ca channel, due to K channel activation is uncertain. It is also uncertain how NO inactivates the Ca channel. In neuronal tissue, increasing cGMP levels or activating cGMP-PK activates, rather than inhibits, Ca current⁹³. We have recently demonstrated that NO inhibits the L-type Ca channel in PA VSM (Fig. 7) and are actively investigating its role in NO-induced vascular relaxation.

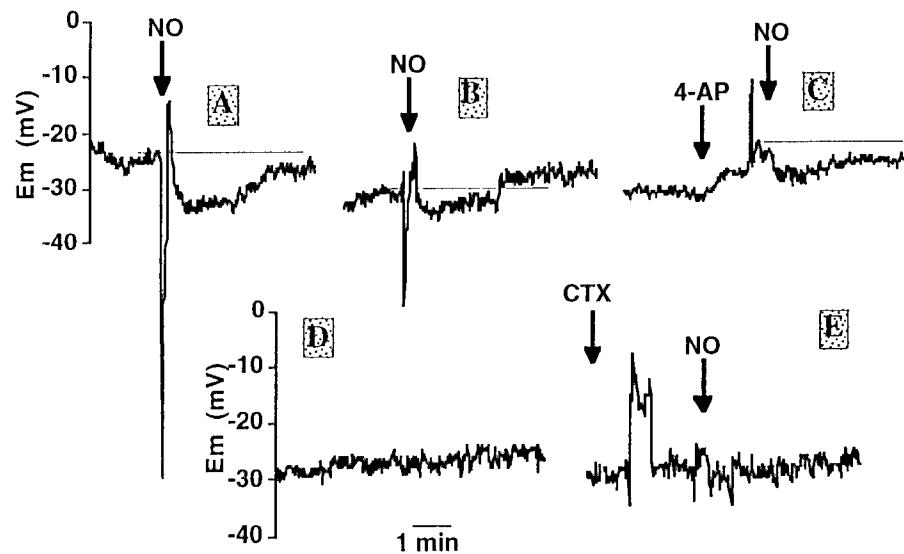


Figure 6 NO hyperpolarizes PA VSM by a charybdotoxin inhibitable mechanism

A. and **B:** NO (1 μ l of 2mM solution) hyperpolarizes pulmonary vascular smooth muscle cell by **C** NO hyperpolarizes cells after depolarization with the K_{DR} inhibitor 4-AP (4-aminopyridine). **D:** Washout period. **E:** Charybdotoxin (CTX) inhibits NO hyperpolarization.

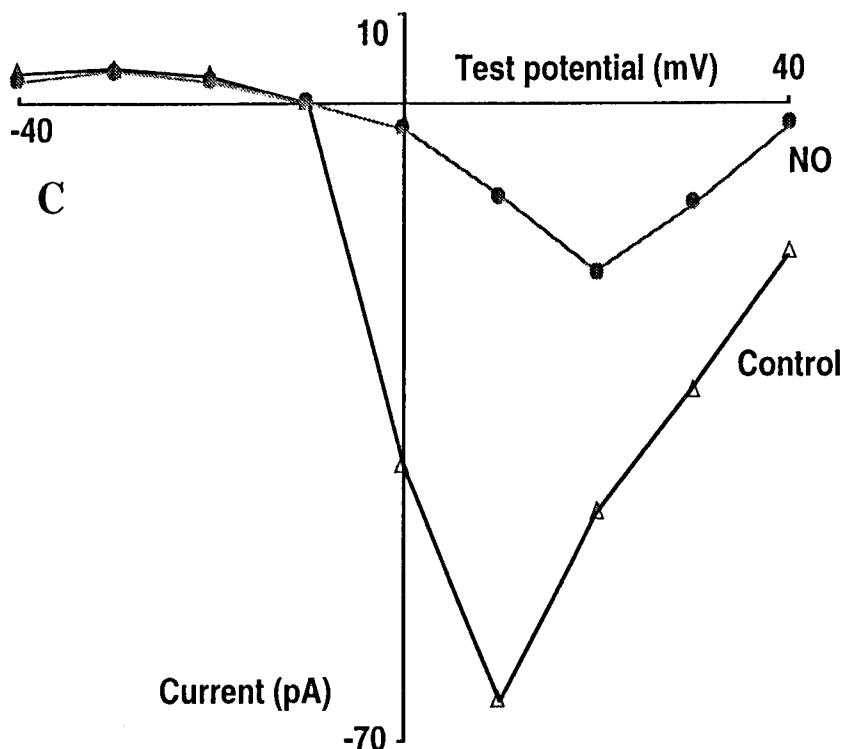


Figure 7 NO inhibits the L-type Ca channel in pulmonary artery vascular smooth muscle cells.
This is a single experiment from a PA VSM cell studied using the amphotericin perforated patch technique.

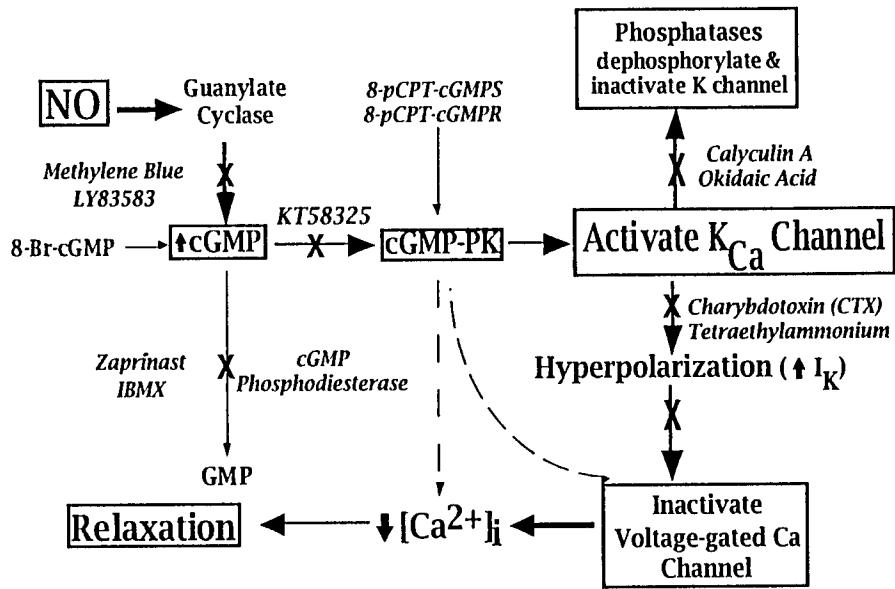


Figure 8 NO causes relaxation of PA VSM in part by activation of K_{Ca} channels and inhibition of the voltage gated Ca channel. NO stimulates guanylate cyclase to produce cGMP, which activates cGMP-dependent protein kinases (cGMP-PK). These kinases phosphorylate and activate K_{Ca} channels, leading to K efflux and membrane hyperpolarization. Hyperpolarization inactivates voltage-gated Ca channels, resulting in smooth muscle relaxation as intracellular calcium levels fall. X depicts inhibition of the pathway by pharmacologic inhibitors. Part of this figure is reproduced with permission from reference 9.

NO/cGMP and Ca sequestration

cGMP lowers $[Ca^{2+}]_i$, at least in part, by promoting Ca sequestration within the SR¹²⁰. Agents which activate cGMP-PK enhance Ca uptake by the SR in PA VSM¹⁰². This occurs as a result of phosphorylation of phospholamban, a Ca transport protein in the SR¹⁰². It is difficult to separate the contributions of K channel activation, Ca channel inhibition and Ca sequestration to NO-induced relaxation as all may occur by a mechanism involving cGMP-dependent activation of cGMP-PK.

Interim summary

NO causes relaxation of PA VSM in part by activation of K_{Ca} channels and inhibition of the voltage gated Ca channel. This mechanism, described in reference 9, is diagrammatically displayed in figure 8.

NO as a regulator of vascular tone

The administration of the NOS antagonist NG-Monomethyl-L-Arginine (L-NMMA) results in systemic hypertension in animals and man^{18,53,54} leading to the conclusion that EDRF plays a major role in the regulation of systemic vascular tone. L-NMMA has no constrictor effect on vascular smooth muscle in the absence of endothelium⁸⁰. Thus, it appears that basal NO production, presumably by the constitutive ecNOS isoform, contributes to the maintenance of normal blood pressure. Mechanical stimuli such as pulsatile flow and shear stress may be responsible for modulating basal release of NO¹⁰⁶.

It is postulated that insufficient NO production is an etiologic factor in essential hypertension. Endothelium-dependent vasodilator responses are decreased in patients with both essential and secondary hypertension^{44,90-92}, whereas endothelium-independent responses are preserved, suggesting that a defect at the level of vascular smooth muscle is not present. Infusion of L-NMMA into the brachial artery caused less vasoconstriction of forearm vasculature in individuals with essential hypertension compared to normal controls⁹¹. Interestingly, L-NMMA infusion in controls resulted in elevation of vascular resistance to values found at baseline in hypertensive patients. These observations suggest that basal NO production may be reduced in essential hypertension. Whether decreased NO production is due to decreased substrate availability, decreased NOS function, or increased destruction of NO remains unknown. Evidence is conflicting regarding the role of decreased substrate in the pathophysiology of hypertension. Intravenous L-arginine, 500 mg/kg, caused prompt vasodilation and reflex tachycardia in hypertensive patients and caused vasodilation in normal controls⁴⁴. However, the authors acknowledged the absence of D-arginine to control for possible nonspecific NO-independent mechanisms. In a second study, intra-arterial L-arginine had no effect on vascular resistance at baseline in hypertensive or control patients⁹⁰. These conflicting data may reflect the different routes of administration and doses of L-arginine.

Although NO is in vogue as a "universal" explanation for many vascular disease, it must be stated that the endothelium produces other vasodilators, such as prostacyclin, as well as constricting factors, such as endothelin. Essential hypertension could be secondary to overproduction of constricting factors relative to relaxing factors. In fact, in the spontaneously hypertensive rat, EDRF is preserved and, instead, excessive constricting factors are present⁷³. Finally, it is possible that endothelial dysfunction is the result, not the cause of essential hypertension. In summary, endothelium-derived NO plays a major regulatory role in the maintenance of resting systemic vascular tone and NO production is deficient in essential hypertension.

NO in the pulmonary circulation

In contrast to the systemic circulation, NO does not appear to modulate resting vascular tone in the normal adult pulmonary circulation. Acute and chronic EDNO inhibition by administration of arginine analogs, at doses which cause systemic hypertension, fail to elevate pulmonary arterial pressure in many species^{10,37,54,87}. These results suggest that the functional importance of basal EDNO production in the adult pulmonary circulation is much less than in the systemic vessels³⁸. However, there is species variation in the importance of NO in basal pulmonary vascular tone^{19,95,110}. Specifically, whereas many animals do not display an increase in basal PVR in response to L-NMMA, children do respond to this NOS inhibitor with segmental pulmonary vasoconstriction¹⁹. Stamler *et al* also noted an increase in PVR in humans treated with L-NMMA (PVR increased 40%) but PA pressure and wedge pressure were unchanged. However, as in the rat, the systemic circulation constricted much more to L-NMMA than the pulmonary circulation (systemic vascular resistance increase 63%)¹¹⁰.

In contrast, under conditions where pulmonary vascular resistance is elevated, EDNO appears to play an important role in vascular regulation. In the fetus, pulmonary vascular tone is markedly elevated under normal conditions and is further augmented following NOS inhibition¹. NO plays a role in the fall in pulmonary vascular

resistance associated with the transition to extrauterine life. NOS inhibition attenuates the decrease in pulmonary vascular resistance associated with delivery and mechanical ventilation with 100% oxygen^{1,25,30}. The importance of EDNO in the regulation of basal pulmonary vascular tone decreases as pulmonary vascular resistance falls during the neonatal period⁹⁴.

Unlike systemic hypertension, pulmonary hypertension does not appear to result from NO deficiency. Instead, pulmonary hypertension may be associated with increased NO production, albeit insufficiently elevated to prevent hypertension⁵⁴. Chronic pharmacological depletion of EDNO does not induce pulmonary hypertension in normoxic or chronically hypoxic rats. Acute administration of arginine analogs produces greater vasoconstriction in isolated lungs of pulmonary hypertensive than healthy rats. More importantly, EDNO production by the isolated perfused lung, undetectable in control rats, is increased in pulmonary hypertensive rats⁵⁴. These observations suggest that basal EDNO synthesis is enhanced in chronic hypoxia, in an attempt to moderate the hypertension. Despite elevated EDNO synthesis in chronic hypoxic rats, PHT is still present, suggesting that the compensatory effects of EDNO are inadequate to restore normal pulmonary hemodynamics.

In summary, EDNO has a smaller role in the basal regulation of low tone adult pulmonary circulation than the systemic vascular bed. In situations where pulmonary vascular tone is increased, the contribution of EDNO becomes significant. Thus, NO may be more important in the fetal than the adult pulmonary circulation. The importance of pulmonary EDNO under normal and pathological conditions, however, needs to be characterized more precisely, particularly in humans.

NO as a neurotransmitter

NO has been documented to participate in neurotransmission within the central and peripheral nervous systems. Glutamate, the major excitatory neurotransmitter in the brain, stimulates cGMP production, among other effects. Glutamate-induced cGMP formation predominately occurs in the cerebellum. Bredt and Snyder postulated and proved that glutamate-stimulated cGMP production in the cerebellum was mediated through production of NO¹⁵. These investigators demonstrated that glutamate and its analogs increased cGMP and L-citrulline levels and that this increase could be prevented by pretreatment with a competitive inhibitor of the enzyme NOS. Furthermore, NOS inhibition could be overcome by the addition of L-arginine, the precursor of NO¹⁵. Subsequently, nNOS has been localized throughout the brain, including the cerebral cortex, hippocampus, cerebellum and brain stem²⁸. The widespread distribution of nNOS among cells of such diverse morphology and function underscores the importance of NO as a mediator of neuronal function.

It is postulated that NO regulates cerebral blood flow during neural activity. The brain possesses a fascinating vascular regulatory mechanism whereby regional increases in neural activity are mirrored by rapid increases in regional perfusion. There is some evidence to suggest that NO is the mediator coupling substrate delivery to metabolic demand in the brain. Several characteristics of NO make it ideally suitable to couple blood flow to neural activity: (1) NO is highly lipophilic and thus diffuses readily across cell membrane;² NOS is present constitutively in brain and can produce NO rapidly in response to an appropriate stimulus; and³ some NOS containing neurons innervate cerebral arteries and possess axon terminals in close proximity to arterioles and capillaries⁴⁶. NOS containing neurons are therefore well positioned to

influence vascular tone. It is attractive to speculate that as NOS containing neurons are stimulated, NO is produced which rapidly diffuses to adjacent vascular smooth muscle, increases cGMP levels and causes vasorelaxation. External electrical stimulation of neural pathways results in cerebral vasodilation which is abolished by topical application of NOS or guanylate cyclase inhibitors⁴⁶. Thus, it appears that NO plays a role in regulating cerebral perfusion during neural activity. However, it should be noted that not all vasodilation in response to neural activity is mediated through NO. Stimulation of various neural pathways produce vasodilation that is not altered by NOS or guanylate cyclase inhibitors⁴⁶, and must be accounted for by mediators other than NO.

In the peripheral nervous system, NO has been shown to participate in the inhibitory innervation of the gastrointestinal tract and may play a role in several pathophysiological states. Motility of the gut is influenced by inhibitory motor neurons innervating intrinsically excitable smooth muscle cells. Within the myenteric plexus, non-adrenergic, non-cholinergic (NANC) nerves inhibit spontaneous contractions of smooth muscle cells of circular muscle and the sphincters of the gastrointestinal tract. While vasoactive intestinal polypeptide was felt to be the major mediator of NANC, recent evidence suggests a significant role for NO. NO, acting as a neurotransmitter, mediates, in part, relaxation induced by inhibitory motor neurons innervating the esophagus, lower esophageal sphincter, pylorus, and colon^{12,17,57,101}. Authentic NO causes inhibition of muscle contraction and membrane hyperpolarization in isolated human colonic circular muscle⁵⁷. Furthermore, spontaneous contractile amplitude is increased in the presence of a competitive inhibitor of NOS, the arginine analog L-N^G-nitro arginine methyl ester (L-NAME). However, NO is not the only mediator of inhibitory motor neurons of the gastrointestinal tract. In several studies, competitive antagonists of NOS only partially block neuronal inhibition or neurally mediated membrane hyperpolarization^{17,57}. Thus NO accounts for some, but not all, neural inhibition of gastrointestinal smooth muscle contractility.

Altered NO production has been implicated in several pathophysiological states such as achalasia⁷⁸, infantile hypertrophic pyloric stenosis¹²² and Hirschprung's disease¹²¹. Achalasia is a disease of esophageal dysmotility characterized by increased resting pressure and impaired relaxation of the lower esophageal sphincter. Mearin et al compared NOS activity in esophageal specimens from patients with and without achalasia⁷⁸. NOS activity, measured by the conversion of radiolabelled L-arginine to L-citrulline, was absent in lower esophageal sphincter specimens from achalasia patients. Immunohistochemical staining, using a polyclonal antibody directed against rat NOS, demonstrated the presence of NOS within the myenteric plexus of control patients and its absence in achalasia patients. Furthermore, isolated, precontracted muscle strips from both affected and unaffected patients relaxed equally well to nitroprusside, suggesting that the altered sphincter tone characteristic of achalasia is due to absence of NOS, rather than an intrinsic abnormality of relaxation.

Infantile hypertrophic pyloric stenosis is a common cause of gastric outlet obstruction in infants. The etiology is felt to be secondary to pylorospasm (e.g. impaired relaxation of the pyloric sphincter). Vanderwinden et al demonstrated the absence of NOS by immunohistochemical staining with NADPH diaphorase in the hypertrophied circular muscle surrounding the pylorus in patients with pyloric stenosis¹²². NADPH diaphorase activity is a redox, colorimetric technique which is a sensitive indicator of NOS activity, but is less specific than *in situ* hybridization techniques.

Their data, nonetheless, suggests that the lack of NOS within the muscular layer accounts for the abnormal relaxation characteristic of pyloric stenosis.

In a separate investigation, Vanderwinden *et al* applied the NADPH diaphorase technique to the enteric nervous system of the distal colon in patients with Hirschprung's disease. Hirschprung's disease is characterized by absence of the myenteric plexus innervating the rectosigmoid colon (aganglionosis), resulting in bowel obstruction in infancy or severe constipation in childhood. NOS was absent in the longitudinal and circular musculature and only weakly present in the myenteric plexus of patients with Hirschprung's disease compared to normal colon¹²¹. Thus, in pathophysiologically distinct processes characterized by abnormal gut motility, NO production is impaired and may contribute to abnormal smooth muscle function.

NO and the immune system

In addition to its role as a neurotransmitter, NO plays a key role in modulating immune responses in man. NO, produced by iNOS in response to cytokines, appears to inhibit bacterial growth and regulate leukocyte adhesion. When activated by cytokines or endotoxin, macrophages release several reactive oxygen species during the "respiratory burst". These radicals are involved in cytotoxicity and cytostasis. Hibbs *et al* was the first to demonstrate that murine macrophage cytotoxicity was dependent upon oxidation of L-arginine⁴⁰. Several groups provided evidence that NO was an integral mediator of macrophage cytotoxicity^{41,74,114}. Stuehr and Nathan demonstrated that it was NO, not the stable oxidation products nitrite (NO_2^-) or nitrate (NO_3^-), that actually inhibited tumor cell DNA synthesis and mitochondrial respiration¹¹⁴. Authentic NO mimicked the cytostatic effects of activated macrophages. In addition, macrophage cytotoxicity was inhibited by NO scavengers, such as reduced myoglobin and superoxide anion. Neither nitrite nor nitrate alone is cytostatic¹¹⁴.

While iNOS was first detected and characterized in murine macrophages, conclusive evidence of the existence of iNOS in human macrophages remains elusive. However, there is indirect evidence for iNOS activity in human monocytes⁶². Interleukin-4, in combination with interferon- γ , stimulate cGMP production in human monocytes. This increase in cGMP is blocked by antagonists of NOS and soluble guanylate cyclase, suggesting a role for NO in stimulating cGMP⁶². The significance of cGMP elevation in monocytes following cytokine stimulation remains unknown.

NO may modulate inflammatory responses by altering leukocyte adhesion. Leukocyte adhesion to the endothelium is an important step in the development and the maintenance of an inflammatory response. Adhesion molecules on the surface of activated leukocytes and endothelial cells allow leukocytes to attach to the vascular wall and emigrate into the interstitial spaces. Kubes *et al* demonstrated that L-NAME caused increased leukocyte adhesion and emigration and that this effect was reversed with L-arginine⁶². Thus, NO may exhibit antiinflammatory properties in addition to its cytostatic role.

NO and insulin secretion

Islet cells of the pancreas sense increases in blood glucose and produce insulin which promotes cellular glucose uptake, thereby maintaining tight control over glucose levels. NO appears to have a role in insulin secretion. iNOS activity has been demonstrated in islet cells and it is postulated that NO mediates islet cell dysfunction

in insulin-dependent diabetes mellitus. Corbett et al measured insulin production in human islet cells following cytokine stimulation²⁴. Glucose-induced insulin production is inhibited by incubation of islet cells with tumor necrosis factor- α , interleukin-1 β and interferon- γ . Although these cytokines act by increasing iNOS activity, inhibition of insulin production was only partially reversed with the NOS antagonist L-NMMA, suggesting that factors other than NO also participate in cytokine-induced insulin inhibition. It is possible that cytokines elicit response in islet cells other than induction of iNOS activity.

NO synthesis in the beta cell is associated with formation of an iron-nitrosyl complex, similar to that produced in bacteria targeted by activated macrophages. The formation of this Fe-nitrosyl complex in the beta cell is prevented by the addition of L-NMMA. Thus, the mechanism of NO-mediated inhibition of insulin secretion may involve destruction of iron-sulfur-containing enzymes, such as mitochondrial electron transport complexes.

Diabetes mellitus is associated with local infiltration of inflammatory cells into the pancreas and destruction of islet cells. The inflammatory cells could serve as a source of NO. It is also possible that cytokines produced by these inflammatory cells induce secondary NO production in islet cells. NO from either source could bind to and inhibit mitochondrial enzymes, resulting in islet cell destruction. These processes may occur in concert with cytotoxic T cell destruction.

NO in sepsis

Sepsis is the human disease state in which the pathophysiologic role for NO is most established^{65,86,98}. Septic shock is associated with activation of the immune system and systemic hypotension. LPS from gram negative bacterial cell walls is a potent stimulator of cytokines and promotes circulatory collapse. Given that both endotoxin and cytokines are stimulators of iNOS, excess NO production has been postulated to account for the severe hypotension characteristic of septic shock. Some evidence suggests that endothelial dysfunction occurs in sepsis and other forms of shock⁶⁹.

Lefer and Lefer compared vasodilator responses to acetylcholine in superior mesenteric artery rings from septic and control rats⁶⁹. The vasodilator response to acetylcholine was reduced approximately 60% compared to control animals. The addition of acidified NaNO₂, which releases NO, caused vasodilation equal to controls. This study suggests that endothelial dysfunction occurs in septic shock and is associated with diminished endothelial dependent relaxation. Others argue that the endothelial dysfunction in septic shock is associated with excessive NO production by cells other than endothelial cells.

Several studies in animals^{85,86} and humans⁹⁸ with sepsis have shown a rapid increase in arterial blood pressure following intravenous administration of NOS antagonists. These data demonstrate that increased NO production occurs in septic shock, although it remains unclear if this represents a protective or pathologic response. Following several case reports of the successful use of NOS antagonists to restore blood pressure septic shock⁹⁷, a randomized double blind placebo-controlled trial was performed. 11 patients with septic shock associated with hypotension were randomized to receive bolus and continuous infusions of L-NMMA or saline⁹⁸. L-NMMA caused rapid and dose-dependent increases in central venous pressure, systemic and

pulmonary arterial pressure and systemic and pulmonary vascular resistance. This implies that the generalized hypotension, characteristic of sepsis, is related to NO-mediated dilation of arterial and venous beds in both the pulmonary and systemic circulation. However, it is not clear that NOS inhibition is in fact beneficial in sepsis. Concomitant with the increase in arterial resistance, a fall in cardiac output occurred in patients receiving L-NMMA⁹⁸, similar to the effect of NOS inhibition in normal animals³⁸. No difference in survival was noted between the treatment groups (2/5 vs. 1/6 in L-NMMA and placebo groups, respectively). Furthermore, no difference in platelet or leukocyte count was apparent between treatment modalities. While this study provides insight into the role of NO in humans with sepsis it raises more questions than answers. Several points are worthy of discussion. First, the decrease in cardiac output associated with L-NMMA may compromise perfusion of organ systems already hypoperfused, particularly the renal and hepatic vascular beds. This is especially problematic as sepsis is associated with impaired oxygen extraction by the peripheral tissues. In addition, a trend toward elevated liver enzymes was noted in the L-NMMA treated group, which may be due to hepatic hypoperfusion or, alternatively, a direct toxic effect on hepatocytes. Furthermore, the increase in pulmonary vascular resistance with L-NMMA administration may prove detrimental. It has been proposed that L-NMMA, in combination with inhalational NO may prove beneficial in the treatment of septic shock⁹⁸. Clearly, the stage is now set for larger studies of the use of NOS inhibitors in sepsis to characterize effects on survival.

NO and the kidney

Evidence that NO has a role in regulating renal blood flow and glomerular function in health and disease is accumulating. The renal mesangial cell is both a producer of and target for NO¹⁰³. NO modulates glomerular filtration rate by regulating afferent and efferent arteriolar tone and controlling filtration coefficient through mesangial cell relaxation¹⁰³. NO regulates cortical and medullary blood flow¹⁶ and participates in tubuloglomerular feedback¹¹⁸ and natriuresis¹⁰⁹. NO inhibits mesangial cell proliferation¹⁰³ which suggests a role for NO deficiency in the progression of glomerulosclerosis.

NO in the breath

NO is present in the breath of humans at part per billion levels^{13,70}. NO levels are highest in the nose and much lower in the alveolus. Several cell types, including pulmonary vascular endothelium, respiratory epithelium and alveolar macrophages, can produce NO, although most breath NO originates from the nose and does not reflect systemic NO production²⁷ (Fig. 1, insert). Nasal NO production is partially inhibited by topical corticosteroids, suggesting that some nasal NO is produced by iNOS. While the exact cellular source of nasal NO remains unknown, inflammatory cells, bacteria or nasal epithelium could contribute to nasal NO²⁷.

Elevated breath NO is present during acute exacerbation of asthma^{4,59,96}, perhaps reflecting inflammation in the small airways. It remains to be seen if other lower respiratory tract inflammatory states, such as pneumonia, are associated with elevated levels of breath NO.

Conclusion

In conclusion, NO is a pluripotent intra- and extra-cellular messenger molecule which regulates a variety of biological processes. NO's capacity to subserve such diverse biologic functions relates to its avid interaction with metal and sulphydryl groups, which are key regulators of the function of most enzymes and proteins. Furthermore, NOS is widely distributed and therefore NO has access to virtually all cells in the body. NO plays a major regulatory role in the physiology of the cardiovascular, neurologic and immune systems.

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CHAPTER 16

HYPOXIA AND NITRIC OXIDE: HOW DOES PHYSIOLOGIC HYPOXIA AFFECT NO SYNTHESIS AND EDRF IN THE PULMONARY VASCULATURE

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INTRODUCTION

In 1980, Furchtgott and Zawadzki first reported that relaxation of rabbit aortic strips in response to acetylcholine requires the presence of an intact endothelium, suggesting the existence of a substance passing from the endothelial cell to vascular smooth muscle. This substance was termed EDRF (endothelium-derived relaxing factor), and shown to relax smooth muscle cells through activation of the soluble form of guanylate cyclase. EDRF has subsequently been identified as nitric oxide (NO) which interacts with the heme component of soluble guanylate cyclase causing an increase in cGMP formation. NO is generated from the nitrogen group of the amino acid, L-arginine through an enzymatic reaction⁵⁸. Several isoforms of NO synthases have now been characterized, which are products of different genes. However, NO synthases are usually classified as either "constitutive" or "inducible". The constitutive form (cNOS) is cytosolic, Ca^{++} and calmodulin dependent, and releases low amounts of NO for short periods in response to receptor and physical stimulation. The NO released by this enzyme acts as a transduction mechanism underlying several physiological responses. It is mainly present in the endothelium and the central nervous system. The inducible form (iNOS) is Ca^{++} and calmodulin independent, and, once expressed, generates NO in large amounts for long periods. It is induced in inflammatory cells but also in other cells such as endothelial and smooth muscle cells in response to endotoxin and some cytokines. NO generated by iNOS mediates part of the inflammatory cell toxicity. The synthesis of NO by both enzymes is stereospecifically inhibited by various L-arginine analogs which act as competitive inhibitors of the NO-synthases, such as L- N_{G} monomethyl arginine (L-NMMA) and L- N_{G} arginine methyl ester (L-NAME), representing methyl and nitro substitutions, respectively, at the guanido nitrogen of L-arginine. The response to NO can also be inhibited or abolished by haemoproteins, methylene blue and superoxide radicals.

In the lung, NO is generated from the constitutive form of NO synthase in pulmonary arterial and venous endothelial cells, nonadrenergic noncholinergic inhibitory neurons, and probably epithelial cells. Cells capable of expressing the inducible enzyme and releasing NO are numerous, including macrophages, neutrophils, mast cells, endothelial and smooth muscle cells, epithelial cells and probably other cell types present in the lung²⁹. So far, the only clearly established role of NO released from these cells after activation with endotoxin and cytokines is as a cytotoxic molecule for invading microorganisms and tumor cells. However, the release of NO via this enzyme may have other undefined biological consequences which may affect lung functions during inflammatory conditions. In the present review, we will focus on the role of NO as a transduction mechanism in the pulmonary vasculature with special attention to changes occurring during chronic hypoxia.

In the pulmonary circulation, NO is normally formed in the endothelial cells by the action of a constitutive form of the enzyme nitric oxide synthase. The endothelial L-arginine/NO pathway can be activated by shear forces exerted by the circulating blood (which in turn causes flow-dependent vasodilation) as well as by receptor-operated mechanisms activated by acetylcholine, bradykinin, substance P, histamine, and platelet-derived products. The fact that NO is released both into the lumen (to inactivate platelets) and away from the lumen (to relax vascular smooth muscle) suggests that it protects against thrombosis and constriction³⁸. The capacity of NO to inhibit proliferation of vascular smooth muscle is a further protective property²⁸. In the normal pulmonary circulation, NO not only mediates vasodilation in response to physical and chemical factors but also opposes vasoconstriction induced by various stimuli such as hypoxia and endothelin^{8,66}. The importance of such a mechanism suggests not only that NO may modulate pulmonary vascular tone but also that impaired NO production may contribute to the development of pulmonary hypertension.

During hypoxic pulmonary hypertension, several studies suggest that one mechanism of altered vascular control is impaired NO-mediated endothelium-dependent relaxation^{4,11,20,54,69}. Loss of NO-mediated pulmonary endothelium-dependent relaxation during hypoxic pulmonary hypertension may contribute to increase pulmonary vascular tone, favor thrombus formation, and facilitate migration and/or proliferation of vascular smooth muscle cells. By increasing hypoxic pulmonary vasoconstriction and therefore mechanical forces exerted on the pulmonary arterial wall, impaired NO production may additionally accelerate the process of vascular remodeling³².

NO-Mediated Endothelium-Dependent Vasodilation During Chronic Hypoxia

Rats exposed to hypoxia for three weeks develop sustained pulmonary artery hypertension associated with structural remodeling of pulmonary vessels^{25,65}. Structural remodeling involves thickening of the blood vessel walls by hypertrophy and hyperplasia of smooth muscle cells and deposition of excessive connective tissue in the adventitia. Smooth muscle appears in the walls of arteries that are not normally muscular and proliferates in the media of previously muscular arteries. All these structural changes lead to a reduction of the lumen of distal pulmonary arteries and a decrease of the normally large cross sectional area of the pulmonary arterial tree^{25,65}.

In isolated lungs from normoxic rats, acetylcholine or ionophore A23187 causes dose-dependent vasodilation in conditions of increased tone by continuous infusion of an endoperoxide analog, U46619⁴. The fact that vasodilation to these substances is

completely abolished by the L-arginine analog, N^G -momethyl L-arginine (L-NMMA), a competitive inhibitor of conversion of L-arginine to NO suggests that NO is the main factor mediating this response. We found that in lungs from rats previously exposed to 3-week normobaric hypoxia, the pulmonary vasodilator response to acetylcholine or ionophore A23187 is completely abolished although response to sodium nitroprusside or linsidomine, two non-endothelium dependent pulmonary vasodilators is not altered in comparison to normoxic rats. These results, which suggest altered NO activity during chronic hypoxia, are in accordance with several studies demonstrating that endothelium-dependent relaxation is impaired in conduit pulmonary arteries during chronic hypoxia. Isolated pulmonary arterial rings from chronically hypoxic rats exhibit less relaxation to acetylcholine or ionophore than those from normoxic animals^{11,54,69}. Relaxation to acetylcholine or adenosine diphosphate was also shown to be attenuated in pulmonary arterial rings obtained from hypoxic patients with chronic obstructive pulmonary disease²⁰. In agreement with an impairment of NO activity during chronic hypoxic pulmonary hypertension, we found that the pulmonary vasoconstrictor response to endothelin is greater in lungs from chronically hypoxic rats than in those from normoxic controls⁴. Moreover, pretreatment with NO-antagonists, which greatly potentiates the vasoconstrictor response to endothelin in lungs from normoxic rats, has no effect in lungs from chronically hypoxic rats. This observation is consistent with the inability of pulmonary vessels from hypoxic rats to oppose endothelin induced vasoconstriction by NO-release and suggests that a defect of NO-synthesis could explain the greater response to endothelin in lungs from hypoxic rats.

Several studies have reported data which may appear in discrepancy with our findings. One group has shown maintained NO-mediated endothelium-dependent vasodilation to arginine vasopressin and ionophore A23187 in U46619 preconstricted lungs from rats previously exposed to 4-week hypobaric hypoxia^{68,70}. Results conflicting with our data may be due to a less severe degree of structural remodeling of the pulmonary vessels in response to hypoxia since in these latter studies, baseline PAP of the isolated lung ventilated in normoxic condition did not differ between normoxic and hypoxic animals. Indeed, we observed that a mild degree of pulmonary hypertension in response to a chronic exposure to 15% instead of 10% FIO_2 , is not associated with impairment of NO-mediated vasodilation in isolated rat lungs. However, other groups reported an increased vasodilator response to substance P or bradykinin in isolated lungs from rats previously exposed to 3-week hypobaric hypoxia³⁸. Apparent discrepancy between these data and ours may be related to testing of different rat strains, use of various ways to increase tone, or use of different vasodilators. In our studies, using slowly infused U46619, the tone was increased to a similar extent in lungs from normoxic and hypoxic animals and endothelium-dependent vasodilators tested at incremental dosage. Moreover, these vasodilators had their action completely blocked in the presence of L-arginine analogs. In the other studies mentioned above, lungs were constricted with acute hypoxia which may constrict more distal arteries than U-46619. Indeed, findings of Barer's group in chronically hypoxic rats suggest that acute hypoxia constricts mostly newly muscularized distal arteries⁷⁷. Moreover, it cannot be excluded that, in particular conditions, endothelium-dependent vasodilation in the pulmonary circulation may involve mediators other than NO⁶.

Several studies also examined the pressor response to acute NO inhibition in lungs from both normoxic and chronically hypoxic rats. In these studies, L-arginine ana-

logs were shown to cause marked vasoconstriction in isolated lungs and isolated pulmonary arteries from chronically hypoxic rats but not in those from normoxic control rats^{7,61}. Concentration of NO decomposition products was also shown to be elevated in the effluent of isolated lungs from chronically hypoxic rats³⁸. These findings were interpreted as evidence of increased basal release of endothelium-derived NO during chronic hypoxia. We also investigated endogenous basal release of NO in the pulmonary vascular wall of normoxic and 4 week hypoxic rats¹⁰. We observed that contractile response to phenylephrine of isolated extralobar pulmonary arteries was similar in normoxic and chronically hypoxic rats when dose response curve was performed shortly after hanging the vessels in the bath (30 to 45 min). However, pulmonary arteries from chronically hypoxic rats exhibited loss of contractile response in comparison to normoxic rats arteries when dose-response was performed after 2 hours of incubation. This loss of responsiveness which became more marked when the incubation time was prolonged further, was observed whether the endothelium was present or not, was restored with the NO-synthesis inhibitor, L-NMMA, and was prevented when incubation was performed in presence of the transcription inhibitor actinomycin D. We also examined the effect of L-NMMA on basal tone. Contraction with L-NMMA was greater in pulmonary arteries from chronically hypoxic rats than in normoxic animals. After prolonged incubation, contraction with L-NMMA was only partially attenuated by endothelial denudation in pulmonary arteries from chronically hypoxic rats, whereas it was completely abolished in rings of normoxic rats. Similarly, when L-NMMA was added to the perfusate only 30 min after isolating the lung, it did not affect baseline pressure nor pressure-flow curve in lungs from normoxic and chronically hypoxic rats. However, when L-NMMA was added 60 min after isolating the lung, it caused a shift in the pressure-flow relationship toward higher pressure in lungs from chronically hypoxic rats but not in lungs from normoxic rats (Fig. 3). This is in accordance with the slowly developing vasoconstriction reported by Oka in isolated lungs from high altitude rats after addition of nitro-L-arginine⁶¹. Therefore, these results suggest that the effect of NO synthesis inhibitors are not related to inhibition of constitutive endothelial NO formation but rather to inhibition of NO synthesis by a newly expressed NO synthase activity in the intima and media of the vessels from hypertensive rat lungs. Recent studies using immunochemistry staining techniques and showing upregulation of NO synthase in the lungs of chronically hypoxic rats are consistent with this hypothesis⁸³. Indeed, in these studies, the monoclonal antibody, which detected the hypoxia-enhanced NO synthase, reacted with both the constitutive and inducible form of NO synthase. In addition, the activity assays used in these studies showed an increase in the soluble NO synthase fraction, consistent with an increase in the inducible NO synthase. Therefore, it is possible that during chronic hypoxia, the endothelial NO synthase becomes downregulated whereas the inducible form is upregulated. This phenomenon is selective for pulmonary arteries since contractile response of aortic rings to phenylephrine are similar in normoxic and chronically hypoxic rats. The consequences of such observations on pulmonary vascular functions remain to be established.

Mechanisms of Impaired NO-Mediated Endothelium-Dependent Vasodilation: Effects of L-Arginine

Potential mechanisms to explain loss of endothelial dependent relaxation in chronic hypoxic pulmonary hypertension include: 1) inability of the vascular smooth muscle

to relax in response to NO; 2) presence of a functional or mechanical barrier that limits transport of NO from the endothelium to the vascular smooth muscle; and 3) impaired synthesis or release of NO. Our finding that NO donors such as Sin-1 or sodium nitroprusside elicit similar dose-dependent vasodilation in lungs from both normoxic and hypoxic rats suggests that the response of smooth muscle to NO is not altered by chronic hypoxia^{4,11,21}. However, studies examining guanylate cyclase activity in pulmonary vascular smooth muscle found that exposure to hypoxia could impair soluble guanylate cyclase-mediated pulmonary arterial relaxation^{15,78}. Reduced vasodilator responses to NO and sodium nitroprusside have also been reported in pulmonary arteries from rats exposed to 10 day hypoxia⁵⁴. In contrast, we found that linsidomine, an endothelium-independent vasodilator agent acting directly on vascular smooth muscle after conversion to NO, induced similar relaxation in large conduit pulmonary arteries from normoxic and 3-week hypoxic rats¹¹. It is also unlikely that loss of endothelium-dependent vasodilation may be ascribed to a mechanical barrier, impairing diffusion of NO from endothelial cells to smooth muscle. Indeed, whereas vascular remodeling associated with hypoxic pulmonary hypertension requires several weeks to reverse after return to normoxia, we found full restoration of the vasodilatory response to acetylcholine or ionophore A23187 after only 48 h of return to normoxia. Loss of endothelium-dependent relaxation does not appear to be due to destruction of NO by oxygen-derived radicals since neither pretreatment of chronically hypoxic rats with superoxide dismutase before return to normoxia nor addition of superoxide dismutase plus catalase to their lung perfuse restores vasodilation to ionophore A23187. Moreover, there is no impairment of endothelial dependent relaxation in systemic arteries from chronically hypoxic rats exposed to the same procedure of reoxygenation when hanged in oxygenated organ chambers¹¹ (Fig. 4). These observations in concert suggest that impaired synthesis or release of EDRF is a likely mechanism to explain the loss of vasodilator response to endothelium-dependent substances in hypoxic pulmonary hypertension.

The hypothesis of a defective release of NO is further supported by the fact that in lungs or pulmonary arteries from hypoxic rats, vasodilator response to acetylcholine or A23187 is fully restored after pretreatment with L-arginine, the precursor of endothelium-derived NO whereas D-arginine as well as L-citrulline, L-ornithine or L-arginosuccinic acid are without effect²¹. L-arginine also attenuates the increased pressor response to endothelin-1 in hypoxic rat lungs. Of note, supplementation of L-arginine does not potentiate vasodilator responses to acetylcholine or A23187 in lungs from normoxic rats. It is therefore likely that, in normoxic rat lungs, maximal endothelium-dependent vasodilation that could be evoked with acetylcholine or ionophore A23187 is achieved, so that supplementation of L-arginine cannot further augment vasodilation. These results suggest that the impaired relaxant activity of pulmonary vessels during chronic hypoxia is most likely due to defective release of NO from the endothelium. Our findings are reminiscent of what has been previously observed in systemic vessels from hypercholesterolemic humans or animals. In those studies, the vasodilator response to acetylcholine is normalized by acute administration of L-arginine¹⁴. It is unlikely that loss of NO-mediated activity during chronic hypoxia can be explained simply on the basis of L-arginine depletion. Indeed, there is no support for a depletion of L-arginine while evaluating plasma or tissue concentrations during chronic hypoxia. Pulmonary arterial tissue concentrations of L-arginine are similar in chronically hypoxic and normoxic rats. Nevertheless, we cannot exclude the possibil-

ity that chronic hypoxia may result in diminished availability of L-arginine in some endogenous pool in close proximity of NO synthase. Another possible mechanism of impaired synthesis or release of NO in chronic hypoxic pulmonary hypertension is direct depression of NO synthase by hypoxia. In support of this, conditions of severe hypoxia depress NO-synthase activity in various *in vitro* models, supporting the importance of O₂ as a cofactor for NO-synthesis^{17,43,58,79}. However, in most of these studies, conditions of anoxia rather than hypoxia were required to depress NO synthase activity. In fact, hypoxia may appear as a stimulating factor for NO synthesis and release. In systemic endothelial cells, hypoxia has been shown to increase intracellular calcium thereby stimulating the synthesis of NO⁹. Moreover, in isolated rat lungs, acute hypoxia stimulates NO production and release^{5,8,52}. More recently, moderate hypoxia (10 to 4 % FIO₂) was shown to cause endothelium-dependent contraction in porcine proximal pulmonary arteries but to stimulate NO and prostacyclin release in distal arteries⁵⁰. In chronically hypoxic rats, endothelium-dependent relaxation is impaired in proximal pulmonary arteries but not in aorta although exposed *in vivo* to a similar level of hypoxia¹¹. It is therefore unlikely that hypoxia per se explains the impairment of NO synthase activity. Recently, nitric oxide synthase gene expression was evaluated in whole lung preparations from chronically hypoxic rats with no apparent alterations⁴⁰. However, exposure of human endothelial cells to hypoxia has recently been shown to inhibit expression of constitutive NO synthase via transcriptional and posttranscriptional mechanisms⁵⁶. These results are consistent with data obtained from histochemical studies showing decreased NO-synthase immunoreactivity in pulmonary arteries from patients with chronic lung diseases³⁰. It is therefore possible that a down regulation of endothelial NO-synthase gene expression occurs during hypoxic pulmonary hypertension.

Acute and Chronic Blockade of NO Synthesis in Normoxic and Hypoxic Animals by L-Arginine Analogs

In the past, it has been hypothesized that maintenance of a low vascular tone in the pulmonary circulation during normoxia was related to some vasodilator substance. To examine the potential role of endothelium-derived NO, experiments with acute and chronic blockade of NO-synthesis have been performed. Various results have been obtained according to species. Acute *in vivo* blockade of NO synthesis during normoxia in dogs, lambs and rats causes increase of systemic artery pressure, decrease of cardiac output but no change in pulmonary artery pressure^{23,35,48}. Similar findings have been reported in humans⁷¹. In such a situation, increase in calculated pulmonary resistance does not prove an active change in resistive properties of the pulmonary vascular bed and can be a passive consequence of the decrease in flow. In isolated lungs from rats and dogs, numerous studies have also demonstrated that acute inhibition of NO synthesis by addition of L-arginine analogs in the perfusate has no effect on the basal tone^{5,37,52,60}. By contrast, in the open-chest rabbit⁶³ and the left lower lobe of the cat⁵⁵, acute blockade of NO causes increase in basal pulmonary vascular resistance. In conscious sheep, basal release of NO may also contribute to the low pulmonary vascular tone at rest since NO synthase inhibition causes increase of pulmonary artery pressure despite concomitant decrease of cardiac output⁴⁴. In this species, potent pulmonary vasoconstriction is elicited by combined inhibition of NO synthase and beta adrenergic blockade during exercise, suggesting that both mechanisms oppose alpha-mediated pulmonary vasoconstriction in exercising sheep⁴⁴. Flow-

induced release of NO has also been demonstrated in isolated lungs from humans, sheep, and dogs^{16,34}. It is therefore possible that endogenous NO plays a role in modulating resting pulmonary vascular tone, however, this may depend on species and possibly, on physiological conditions.

In contrast to such divergent observations during normoxic conditions, there are consistent findings to indicate that endothelium derived NO plays a significant role in modulating pulmonary vascular tone during acute hypoxia. Numerous investigators have reported potentiation of acute hypoxic vasoconstriction by NO synthase inhibitors in isolated lungs from rats^{5,52,61} and dogs³⁴ as well as in intact animals⁶¹. To elucidate further the role of endogenous NO in the normal and chronically hypoxic pulmonary circulation, chronic blockade of NO synthase has been performed in rats maintained in room air or exposed to an hypoxic environment^{35,48}. After 2 to 4 weeks of L-NAME administration in drinking water (1.85 mM) or by gavage (50 mg/kg once a day), there was a significant increase in systemic artery pressure but no significant change of pulmonary artery pressure, and a significant hypertrophy of the left ventricle without alteration of the right ventricular weight as compared to control rats receiving the solvent alone and maintained in the same environment conditions. Moreover, structural remodeling as evidenced by degree of muscularization of distal vessels as well as medial thickness of muscular arteries was not affected by L-NAME administration. Therefore chronic blockade of NO synthesis does not cause pulmonary hypertension in rats maintained in a normoxic environment nor does it aggravate pulmonary hypertension during exposure to chronic hypoxia. These data may be interpreted as suggesting that NO does not play an important role in the normoxic pulmonary circulation. However, it cannot be excluded that the marked decrease in cardiac output observed during chronic blockade of NO synthase masks stimulation of structural remodeling in response to inhibition of NO synthesis. The role of hemodynamic forces in the remodeling of pulmonary arteries during hypoxia is suggested by experiments demonstrating attenuation of vascular wall thickening distal to a coarctation of the left pulmonary artery in rats and calves exposed to chronic hypoxia^{64,73}. Therefore L-NAME's lack of effect on pulmonary vascular morphology does not rule out the possibility that endogenous NO plays a significant role in maintaining smooth muscle quiescent in the pulmonary vascular wall.

NO and Pulmonary Vascular Reactivity during Normoxia and Chronic Hypoxia

If NO-mediated endothelium dependent relaxation is adversely affected by chronic hypoxia in pulmonary vessels, this would theoretically lead to an increased tone and to an exaggerated or inappropriate vasoconstrictor response to various stimuli. Because NO is also a potent inhibitor of platelet adhesion and aggregation, such a defect in NO production might also favor platelet aggregation and, ultimately, thrombosis. A vicious circle may thus be created since the release of platelet-derived substances such as platelet-activating factor, serotonin, and noradrenaline, which induce vasodilation through the release of NO, may lead to further vasoconstriction and platelet adhesion in the absence of protective endothelial formation of NO. Additionally, the pulmonary vascular action of various circulating substances or neurotransmitters may become altered during chronic hypoxia⁵⁵. For example, serotonin originating from the gut is cleared from the blood stream partly by the liver, and partly by the pulmonary endothelium. While low doses of serotonin induce pulmonary vasodilation in

lungs from normoxic rats, no vasodilatory action of the amine can be shown in lungs from rats previously exposed to chronic hypoxia (unpublished results). Similarly, neurotransmitters released from cholinergic or sensory nerve endings such as acetylcholine, substance P or calcitonin gene-related peptide have a decreased ability to induce pulmonary vasodilation in rats exposed to chronic hypoxia. This suggests that neuromediators may exert different effects on tone depending on whether they are released during normoxia or chronic hypoxia⁵⁵.

In addition to promoting pulmonary vasodilation, NO can also attenuate vasoconstriction induced by pharmacological or physiological stimuli. For example, the role of endothelial NO formation in limiting the degree of pulmonary vasoconstriction induced by acute hypoxia has been extensively investigated and such studies are described in the chapter by Stephen Archer in this book. In isolated perfused rat lungs, NO inhibitors such as NDGA, hydroquinone, methylene blue and L-arginine analogs have all been documented to potentiate hypoxic pulmonary vasoconstriction^{5,8,52}. In these experiments, because NO inhibition during normoxic ventilation did not cause alteration of basal pulmonary artery pressure, accentuation of the hypoxic pressor response by NO inhibition was interpreted as NO release during acute hypoxia.

Inhibitors of NO not only potentiate hypoxic pulmonary vasoconstriction but also enhance the pressor response to substances such as endothelin or serotonin when these substances are used at concentrations which induce vasoconstriction^{4,21}. Receptors to serotonin and endothelin have been shown at both the endothelial and smooth muscle cell levels suggesting that their vasoconstrictor action is modulated by simultaneous activation of endothelial cells which in turn release NO⁵³. Therefore, in lungs from chronically hypoxic rats, the vasoconstrictor responses to serotonin or to endothelin are greater than those from normoxic controls^{4,21,22}. Moreover, pretreatment with NO inhibitors, which greatly potentiates their vasoconstrictor actions in lungs from normoxic rats, has no effect in lungs from chronically hypoxic rats^{4,21}. These observations are consistent with the inability of pulmonary vessels from hypoxic rats to oppose various vasoconstrictor stimuli by the release of NO and suggest that the defect of NO production could explain the greater response to substances such as endothelin or serotonin in lungs from chronically hypoxic rats. An exaggerated response to vasoconstrictor stimuli in hypoxic rat lungs could also result from an increased wall to lumen ratio of the hypertrophied pulmonary arteries. Such a possibility is unlikely since the pressor responses to these agonists are restored to normal levels after only 48 hours of return to normoxia. This interval is too short for reversing the structural changes of the vessel wall²⁵.

Therefore, the loss of NO-mediated pulmonary endothelium-dependent relaxation during experimental hypoxic pulmonary hypertension may prove important for the local regulation of pulmonary vascular tone and may facilitate vasoconstriction arising from various stimuli. It is not clear presently whether such defect in NO production may also lead to enhanced synthesis of vasoconstrictors such as endothelin which appear to be under the control of NO, possibly via a cGMP-mediated mechanism⁴⁷.

Effects of Continuous Inhalation of NO During Chronic Hypoxia

As previously emphasized, loss of endothelium-derived NO formation during chronic hypoxia could lead to an increased tone and to an exaggerated or inappropriate vasoconstrictor response to various stimuli. In addition, NO may also be viewed as a loss of one of the mechanisms which maintains smooth muscle cells quiescent in the

pulmonary circulation. In rats exposed to chronic hypoxia, we used inhaled NO as a substitute for endogenous NO in the pulmonary circulation⁴⁹. Recent data have shown that inhaled NO which reaches the pulmonary vessels through an abluminal route, induces potent pulmonary vasodilation²⁶. Since NO is rapidly inactivated by combining with hemoglobin, vasodilation is restricted to the pulmonary vascular bed with no change in systemic arterial tone. Indeed, in sheep and humans exposed to acute hypoxia, NO inhalation is associated with a dose-dependent selective pulmonary vasodilatory effect^{26,27}.

The short term effects of inhaled NO were assessed in chronically hypoxic conscious instrumented rats. Acute inhalation of NO induces potent and selective pulmonary vasodilation in rats previously exposed to 3-week hypoxia and studied in an hypoxic environment. The vasodilator effect of NO occurs in a dose-dependent manner in response to concentrations varying from 5 to 40 ppm. Pulmonary vasodilation is rapid, maximal within 3-5 min, and is maintained throughout the inhalation period. NO inhalation does not alter cardiac output or systemic artery pressure. Discontinuation of NO inhalation is associated with a rapid rise in pulmonary artery pressure which returns to its control value within 2 to 3 minutes.

In contrast to chronically hypoxic rats, normoxic rats do not exhibit pulmonary vasodilation in response to inhaled NO. Similarly, Frostell *et al* did not observe any reduction of baseline normal pulmonary artery pressure in sheep during NO inhalation²⁶. These results are consistent with the fact that the pulmonary vascular tone is elevated in rats with chronic hypoxic pulmonary hypertension but minimal in normoxic control animals.

The development and maintenance of pulmonary hypertension during chronic hypoxia is the result of increased vascular tone, polycythemia and structural remodeling of pulmonary arteries. Continuous inhalation of NO during exposure to hypoxia attenuated right ventricular hypertrophy while it did not change the hematocrit. Since right ventricular hypertrophy is the consequence of sustained pulmonary hypertension, these results suggest that NO had a sustained lowering effect on pulmonary artery pressure. The development of hypoxic pulmonary hypertension is associated with hypertrophy and hyperplasia of smooth muscle cells in normally muscularized arteries and the appearance of new smooth muscle cells in non-muscular and partially muscularized segments of the intraacinar circulation⁶⁵. Concomitant to the lesser degree of right ventricular hypertrophy, muscularization of distal pulmonary arteries at alveolar duct and wall levels although still significant in comparison with the normoxic group, was less severe in hypoxic rats subjected to NO inhalation. Inhalation of NO, therefore, partially prevented pulmonary vascular remodeling caused by hypoxia. There are several mechanisms by which inhaled NO could protect against vascular remodeling.

Vasodilation

As a vasodilator, NO limits the increased mechanical forces exerted on the pulmonary endothelium during hypoxic pulmonary vasoconstriction. It is now well known that an important stimulating component of endothelial NO-synthase activity is shear stress exerted on the vascular wall¹⁸. Because shear stress is directly proportional to flow rate and inversely proportional to the cube of the vessel radius, the increased formation of NO in response to shear stress may be viewed as an adaptative mechanism to normalize increased wall shear stress associated with prolonged vasocon-

striction. Because elevated tensile wall stress induces thickening of the vessel wall^{32,36,67,75}, endothelial NO formation in response to shear stress may also be viewed as a regulatory mechanism of vascular remodeling. In cases of chronic hypoxic pulmonary hypertension, impaired NO synthesis, which favors vasoconstriction, may indirectly contribute to enhance arterial thickening due to increased mechanical forces exerted on the pulmonary vascular wall^{64,73}. The fact that inhaled NO prevents pulmonary hypertension in chronically hypoxic rats⁴⁹ by preventing the vasoconstrictor component of hypoxic pulmonary hypertension, inhaled NO could indirectly attenuate the remodeling of the pulmonary arterial wall. This mechanism is also suggested by the observation that various vasodilator agents acting on tone through different mechanisms, such as calcium antagonists⁷², methyldopa⁷⁴, or atrial natriuretic peptide⁴² have also been shown to decrease medial thickening of pulmonary arteries in chronically hypoxic rats.

Growth Inhibition

Because of its direct antiproliferative properties, NO may also oppose smooth muscle cell proliferation and protect against vascular remodeling and development of pulmonary hypertension. Indeed, various chemically dissimilar NO-generating drugs have been shown to inhibit DNA synthesis and vascular smooth muscle cell proliferation *in vitro*^{28,45,59}. This effect is shared by 8-bromo-cGMP, suggesting that the relaxant action of NO is mediated by cGMP as the second messenger. However, because high cGMP elevations and concentrations of NO-generating drugs are needed to produce these effects, it has been questioned whether endogenous NO could behave as a growth inhibitor during *in vivo* conditions. On the other hand, vascular smooth muscle cells in culture have been shown to not fully express cGMP dependent protein-kinase, suggesting that observations made in cultured cells may not apply to *in vivo* conditions⁵¹. Therefore, attenuation of muscularization of distal pulmonary arteries from chronically hypoxic rats in response to inhalation of NO at low concentrations may not be related with certainty to a direct inhibitory effect of NO on smooth muscle cell growth.

In addition to its growth-inhibitory properties, NO may also interfere with endothelial production of vasoconstrictor and growth-promoting substances. Recently, NO has been shown to inhibit hypoxia-induced expression and production of endothelin-1 and PDGF-B chain in endothelial cells⁴⁷. Although such an effect of NO has not been demonstrated in *in vivo* models, it is possible that impaired synthesis of NO could lead to increased expression of vasoconstrictors and growth factors during conditions of endothelial dysfunction such as that observed during chronic hypoxic pulmonary hypertension.

Clinical Aspects, Patients with Chronic Hypoxic Pulmonary Hypertension

Several reports now suggest that pulmonary endothelial dysfunction and abnormal vascular response occur in various forms of human pulmonary hypertension. Decreased production of prostacyclin or NO and increased release of endothelin have recently been described in patients with primary or secondary pulmonary hypertension^{12,20,31}. In some secondary forms of pulmonary hypertension, endothelial-cell dysfunction may be produced by the process initiating the disorder, such as the increased shear and mechanical injury associated with left to right shunt and increased pulmonary blood flow. In patients with the Eisenmenger's syndrome, impaired NO produc-

tion may result from these mechanical alterations¹⁹. In patients with pulmonary hypertension complicating the course of chronic hypoxic lung disease, a variety of environmental stimuli may also, individually or in combination, contribute to promote endothelial dysfunction and injury. In patients with chronic hypoxic lung disease (COLD), hypoxia, acidosis, lung inflammatory processes, reduction of the pulmonary vascular bed through parenchymal destruction, increased shear forces associated with increased pulmonary blood flow and polycythemia may through different ways affect endothelial function and/or NO production or release. Chronic hypoxia may also be an important factor, as long-term oxygen therapy reverses or at least impedes the progressive development of pulmonary hypertension. However, pulmonary hypertension is rarely severe in such patients, and endothelial dysfunction, including impaired synthesis of NO may not be as marked as in hypoxic animal models⁸².

In vitro studies of pulmonary arteries obtained from patients at the time of their heart-lung transplantation (for end-stage chronic obstructive lung disease (COLD)) compared with pulmonary arteries from patients undergoing lung resection for cancer have provided some insight into the effects of chronic lung disease on endothelial release of NO²⁰. In pulmonary arteries from patients with COLD, relaxation to acetylcholine and adenosine-diphosphate was found to be impaired as compared to subjects without evidence of chronic lung disease. This impaired relaxation was ascribed to reduced NO synthesis and/or release since vasodilator response to NO donors such as sodium-nitroprusside was maintained. Rings from COLD patients also demonstrated an exaggerated contractile response to the alpha-adrenergic agonist phenylephrine, this was taken as evidence that release of NO which attenuated the vasoconstrictor effects of phenylephrine in control rings was lacking in rings from COLD patients. Interestingly, a positive correlation was found between the extent of intimal thickening and the impaired release of NO. Moreover, there was a positive correlation between relaxation and the partial pressure of arterial O₂ measured before transplantation. Taken together, these observations go some way towards implicating impaired NO release and the structural changes of the blood vessels. Moreover, they suggest that the degree of hypoxemia as a reflection of the severity of the underlying lung disease may be related to the impaired production of NO.

In recent studies performed in patients with COLD of lesser severity and studied during hemodynamic investigation, pulmonary vasodilation induced by various doses of acetylcholine was compared to that induced by increasing concentrations of inhaled NO¹. Acetylcholine which is a potent endothelium-dependent vasodilator agent in the systemic circulation is also a potent pulmonary vasodilator which may be used to detect functional abnormalities of the pulmonary endothelium⁷⁶. In human diseases such as atherosclerosis or systemic hypertension, the impaired ability of acetylcholine to induce systemic vasodilation has been ascribed to endothelial dysfunction with impaired synthesis and/or release of NO⁶². In patients with COLD, acetylcholine induces a dose-dependent decrease of pulmonary artery pressure and a slight reduction of systemic arterial pressure with concomitant increase of cardiac output. In contrast, inhaled NO induces a selective concentration-dependent decrease of pulmonary artery pressure with no change in cardiac index. Although pulmonary artery pressure decreases less in response to i.v. acetylcholine than in response to inhaled NO, pulmonary vascular resistance decreases by a similar magnitude in response to both agents. These results therefore suggest that endothelium-dependent vasodilator response to acetylcholine is not greatly impaired in most patients with COLD. However, pulmo-

nary vasodilation does not appear to be a consistent response to acetylcholine in patients with COLD. In some patients, acetylcholine infusion fails to cause pulmonary vasodilation, suggesting inadequacy of significant NO production in their pulmonary endothelium. Consistent with these observations, recent histochemical studies performed on pulmonary vessels from patients with various forms of pulmonary hypertension suggest disparity of endothelium NO synthesis among patients³⁰. While in normal human lungs, strong endothelial cell immunostaining was observed for endothelial nitric oxide synthase, the intensity of endothelial cell immunostaining varied from one case to another in patients with chronic lung disease and pulmonary hypertension. In contrast, there was little or no NOS-immunoreactivity over the vascular endothelium of remodelled pulmonary arteries in patients with primary pulmonary hypertension.

Polycythemia and NO-Mediated Vasodilation

Polycythemia is an important contributing factor to the increase of pulmonary artery pressure and central blood volume in chronically hypoxic animals as well as in patients with chronic hypoxic lung disease. In chronically hypoxic rats, restoring a normal hematocrit by hemodilution is associated with a more than 50% decrease of the elevated pulmonary artery pressure^{24,41}. However, it is not clear whether these observations are explained only by rheologic abnormalities resulting from the increased blood viscosity. Important interactions may also exist between hemoglobin concentration and NO-mediated vasodilation, which may interfere with vascular reactivity^{39,46}. On one hand, polycythemia may contribute to enhance basal release of NO by increasing blood viscosity and shear stress (which is directly related to blood viscosity), but on the other hand, polycythemia could inactivate NO because of the buffering activity of hemoglobin^{39,46,80,81}.

Using acetylcholine as a screening agent for pulmonary vasodilator response, we investigated a large population of patients with COLD and found that acetylcholine failed to induce pulmonary vasodilation in patients with polycythemia³. Moreover, we found a negative correlation between hemoglobin concentration and pulmonary vasodilation as reflected by the fall in resistance and the increase in cardiac output in this study population. In a subgroup of patients whose hemoglobin level was above 15.5 g/100 ml, we examined the response to acetylcholine infusion before and after hemodilution. While no vasodilator response to acetylcholine was observed during basal conditions, either in the pulmonary or systemic circulation, a decrease in both pulmonary and systemic vascular resistance occurred after hemodilution in response to acetylcholine, suggesting impairment of NO-mediated vasodilation associated with polycythemia.

In complementary studies, we investigated another group of patients and examined the effect on forearm blood flow of agonist-stimulated release of NO using acetylcholine and basal release of NO using the NO-synthase blocker LMMA². Patients were classified according to their hemoglobin levels, whether less or greater than 15.5 g/100 ml. Drugs were infused locally into the brachial artery and forearm blood flow was measured using venous occlusion plethysmography. Again, we found that patients with polycythemia did not respond to acetylcholine by increasing their forearm blood flow whereas they demonstrated a greater vasoconstrictor response to LMMA. Polycythemia may therefore contribute to enhance basal NO release through direct activation of NO-synthase activity, preventing further pharmacological stimu-

lation by acetylcholine. Inactivation of NO by hemoglobin may also occur to limit the vasodilating potency of NO resulting from both shear stress- and agonist-induced stimulation of NO-synthase activity³⁹.

NO and Pathogenesis of Hypoxic Pulmonary Hypertension

An important question raised by these observations is whether impaired NO formation by pulmonary endothelial cells represents a primary event or may be secondary to the pulmonary hypertensive disease process. It may be speculated that impaired release of NO is a causative factor for chronic hypoxic pulmonary hypertension. The observation that, in systemic arteries, removal of the endothelium is followed by proliferation of the underlying vascular smooth muscle cells, is consistent with such an hypothesis^{13,33}. Moreover, the protective effects of NO inhalation against pulmonary vascular remodeling are consistent with those recently obtained in hypercholesterolemic rabbits using supplementation of dietary L-arginine to improve endothelium-dependent relaxation of systemic arteries¹⁴. In both studies, supplying NO chronically was associated with an improvement in structural changes induced by the causal disease.

However, several arguments suggest that impaired synthesis of NO may occur as a secondary event during the course of pulmonary hypertension. While abnormal features in pulmonary arterial structure are known to develop progressively, and pulmonary artery pressure to increase slowly during continuous exposure to hypoxia⁶⁵, some morphological changes appear as early as after three days of exposure to hypoxia including intimal and subendothelial thickening⁵⁷. The occurrence of rapid structural changes is also suggested by experiments performed in isolated pulmonary artery segments from rats showing that pressure-induced connective tissue synthesis occurs within four hours⁷⁵. These observations contrast with the fact that NO-mediated endothelium-dependent vasodilation is only slightly impaired in lungs from one week hypoxic rats but become abolished after 15 days of hypoxia⁴. Impairment of endothelial NO production therefore may not represent an early defect during development of hypoxic pulmonary hypertension. Hence, dysfunction of the L-arginine pathway is likely to be a secondary event involved in the maintenance rather than in the initiation of hypertension. Whatever the cause or the consequence of hypoxic pulmonary hypertension, impairment of the constitutive NO-vasodilator system may play an important role in the maintenance and the worsening of the pulmonary hypertensive process.

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CHAPTER 17

NITRIC OXIDE AND THE HYPOXIC NEWBORN

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INTRODUCTION

Few settings exist in biology which parallel the dramatic and rapid physiologic adjustments that must occur during adaptation of the fetus to postnatal life. Not only does the normal fetus tolerate low oxygen tensions (PO_2) at levels which cause asphyxia in adults, but it thrives and grows, while continuing its preparation for postnatal life. Several compensatory mechanisms exist in fetal life which provide important adaptive skills to allow it to tolerate these "hypoxic" conditions, such as increased oxygen affinity of fetal hemoglobin, relatively high organ blood flow, elevated hematocrit, and unique mechanisms for transplacental oxygen transfer^{13,28,59,60,64,76}. Although arterial oxygen tension is low in the normally oxygenated fetus, there is a narrow safety margin and PO_2 plays an important vasoregulatory role *in utero*. In fact, patency of the ductus arteriosus and high pulmonary vascular resistance are at least partly due to low PO_2 in the normal fetus. Yet, the fetus remains susceptible to hypoxia; decreasing fetal PO_2 by 5–10 torr markedly compromises fetal well-being, leading to tissue oxygen deprivation with lactic acidosis, and can cause fetal demise in the absence of successful adaptive responses^{59,64}. Adaptive hemodynamic responses to acute hypoxia in the fetus include redistribution of cardiac output which favors perfusion of the placenta to sustain fetal oxygenation and increased blood flow to "essential organs," such as the brain, heart, and adrenals, at the expense of "nonessential" organs, including the lungs^{23,68,69,71}. The vital importance of this stress response is illustrated by studies in which interruption of adaptation exacerbates hypoxia and increases metabolic acidosis⁷¹. With severe or prolonged intrauterine stress, adaptive responses may become "maladaptive," leading to sequelae such as intrauterine growth retardation, abnormalities in adaption at birth and postnatal asphyxia.

Although low PO_2 may be tolerable *in utero*, increased oxygenation is necessary for the dramatic hemodynamic changes which are required for successful adaption at birth, such as closure of the ductus arteriosus and the marked drop in PVR⁷⁸. Mechanisms underlying transition of the lung circulation at birth are incompletely understood, but include establishment of a gas-liquid interface, increased PO_2 , rhythmic

distension of the lung, shear stress, and altered production of vasoactive mediators^{4,12,18,19,24,26-29,31,51,52,85}. In some newborns, however, these mechanisms fail to achieve or sustain this decrease in PVR, leading to severe hypoxemia due to continued shunting of blood flow across the ductus arteriosus or foramen ovale. Clinically associated with a syndrome referred to as persistent pulmonary hypertension of the newborn (PPHN), this pathophysiology occurs with diverse cardiopulmonary abnormalities, and leads to significant morbidity and mortality^{54,82}. Although most commonly recognized in term neonates, elevated PVR and abnormal vasoreactivity is also associated with severe respiratory distress syndrome (RDS) in premature neonates^{22,87}.

Current approaches in the management of hypoxic neonates with PPHN and premature infants with severe RDS are partly limited by the lack of insight into basic mechanisms underlying the pulmonary vascular abnormalities in these conditions. Although the pathogenesis and pathophysiology of PPHN are poorly understood, recent experimental studies have examined the hypothesis that abnormal endothelial function contributes to pulmonary vascular abnormalities in the perinatal lung. More than a simple barrier, endothelial cells can regulate vascular tone by releasing an array of vasoactive products, including endothelium-derived relaxing factor (EDRF) or nitric oxide (NO), prostacyclin (Pg 12), endothelium-derived hyperpolarizing factor, endothelin-1 (ET-1), and others. These products have been shown to contribute substantially to vascular tone and growth in many experimental and clinical settings^{61,79,83,84}. Until recently, however, few studies had examined maturational changes in endothelial function and potential roles of endothelial products, especially NO, in the perinatal lung circulation.

The purpose of this chapter is to review recent studies examining the roles of endogenous nitric oxide (NO) in regulation of vascular tone in the developing lung, including its presence *in utero*, its role in the normal decline in PVR at birth, and abnormalities in NO activity in experimental models of PPHN and RDS. The potential role of inhaled NO therapy in the management of the hypoxic neonate is also discussed.

Role of Nitric Oxide in Regulation of Perinatal Pulmonary Vascular Tone:

Fetal Lung Circulation:

Pulmonary artery pressure is high and blood flow is low in the normal fetus, with flow to the lung accounting for less than 8% of combined ventricular output during late gestation⁷⁴⁻⁷⁸. High PVR allows for most of the right ventricular output to cross from the main pulmonary artery to the ductus arteriosus into the descending aorta. As a result, more blood flow is directed to the site of gas exchange, the placenta, enhancing fetal oxygenation and substrate uptake. Mechanisms which maintain high PVR *in utero* include various mechanical and biochemical stimuli, such as low PO₂, the lack of a gas-liquid interface, and perhaps a predominant production of vasoconstrictor substances over vasodilators^{28,38}. Increased production of vasoconstrictor substances, such as leukotrienes⁸⁰ and ET-1³⁹, decreased production or activity of vasodilators (such as prostacyclin, NO or others), or altered smooth muscle responses to vasoactive stimuli may contribute to high PVR *in utero*. For example, it has been hypothesized that sulfidopeptide leukotrienes (LTC4 and D4), potent vasoconstrictors, contribute to high PVR in the normal fetus, since infusions of putative leukotriene receptor and synthetic antagonists cause fetal pulmonary vasodilation⁸⁰. These blockers,

however, have nonspecific effects, and whether leukotrienes contribute in part to high fetal PVR remains controversial²¹. Similarly, ET-1, a potent endothelium-derived vasoconstrictor peptide, has been purported to contribute to high basal PVR, since intrapulmonary infusion of a selective ET A receptor antagonist, BQ123, increases fetal pulmonary blood flow³⁹ and augments vasodilation during exposure to increased shear stress. High preproET-1 mRNA content has been found in fetal rat lung⁵⁷ and umbilical cord ET-1 levels are much higher than adult values⁶⁶, further supporting the potential role of ET-1 in maintaining high fetal PVR.

The fetal pulmonary circulation is further characterized by its capacity to oppose vasodilation to some stimuli during prolonged treatment ("time-dependent autoregulation")^{1,8}. Several vasodilator stimuli, including increased PO₂, many pharmacologic agents, and shear stress, increase fetal pulmonary blood flow during acute exposure, but this response is often only transient. Despite continued exposure to these dilator stimuli, blood flow steadily decreases toward baseline with time^{1-3,8-10}. Mechanisms which oppose sustained elevations of blood flow in the fetal lung are unknown, but these findings led to the hypothesis that vasoconstrictor mechanisms exist in the fetus which resist pulmonary vasodilation. Some pharmacologic agents which act by directly stimulating smooth muscle cell relaxation by increasing cyclic guanosine monophosphate (cGMP) (including 8-bromo-GMP, atrial natriuretic peptide and inhaled NO), cause sustained pulmonary vasodilation during prolonged treatment^{2,45}. In contrast, several endothelium-dependent agonists (including acetylcholine, bradykinin, histamine, tolazoline, oxygen, shear stress and others) are unable to sustain vasodilation^{1-3,7,8}. Fetal pulmonary arterial smooth muscle seems responsive to direct-acting vasodilator stimuli during late gestation⁵ and the inability of the endothelial cell to release or sustain release of dilator substances, increased cGMP phosphodiesterase activity⁹¹, or enhanced release of a vasoconstrictor (such as ET-1^{39,40}) may play important roles in autoregulation in the fetal pulmonary circulation^{1,2} (see below). We speculate that persistence of these fetal mechanisms into the neonatal period could potentially increase pulmonary vasoreactivity and tone, and lead to failure of postnatal adaptation.

Recent studies have demonstrated the presence of NOS gene and protein in rat and ovine fetal lungs^{37,67}. To determine whether the endothelium-specific (type III) NOS isoform (eNOS) is present in the developing lung as well as temporal changes in its expression, we performed immunostaining of fetal, neonatal and adult lung tissues using a specific antibody for eNOS³⁷. Immunoreactive eNOS is present throughout fetal life, including lung tissue from as early as 0.3 gestation. eNOS immunostaining remained prominent during the neonatal period, after which there was an apparent decrease in staining intensity with postnatal age. The early presence of NOS led us to speculate that endogenous NO activity may also play a role in angiogenesis in the developing lung³⁷. Interestingly, cultured bovine aortic endothelial cells express 3 to 6-fold increases in eNOS protein and mRNA while growing, but eNOS is diminished when these cells become confluent¹¹. However, since NOS inhibition did not influence endothelial growth, it may serve as a marker for growing cells. Alternatively, NO may serve other roles in this time period, such as limiting muscularization in newly-forming vessels by blocking smooth muscle cell growth, as indirectly suggested by other studies²⁵. Steady state eNOS mRNA levels progressively increase with development in fetal rat lungs, suggesting that eNOS expression is developmentally regulated⁶⁷. Pulmonary vascular surface area dramatically increases during late-

gestation, however, which may account for the apparent increase in eNOS mRNA and protein during fetal life.

Despite the striking presence of NOS protein in the developing lung, *in vitro* studies suggest that fetal pulmonary arteries have diminished NO activity in comparison with postnatal arteries, and that maturational changes in endothelial cell function may contribute to developmental changes in pulmonary vascular reactivity⁵. In contrast, fetal pulmonary vascular smooth muscle appears capable of responding completely to NO early in gestation^{5,44}. The effects of endothelium-dependent and -independent agonists on intralobar pulmonary artery rings were studied in smooth muscle baths⁵. Pulmonary arterial rings from fetal pulmonary arteries showed little relaxation to endothelium-dependent agonists, including acetylcholine, adenosine diphosphate, and A23187. In contrast, the endothelium-independent agent, sodium nitro-prusside (SNP; and NO donor), caused complete relaxation. In comparison with fetal pulmonary artery rings, rings from newborn and adult animals had significantly greater relaxation in response to the endothelium-dependent agonists. SNP-induced relaxations were not different between fetal, newborn and adult rings. Thus, it appears that the ability of developing pulmonary vascular smooth muscle to respond to exogenous NO precedes the immature endothelium's ability to release or sustain release of NO.

Physiologic evidence of lung NOS activity in the late gestation fetus has been reported⁴, and basal NO activity appears to modulate PVR in the ovine fetus at least as early as 115 days (0.78 term)⁴⁴. Endothelium-dependent drugs, such as acetylcholine, bradykinin, and histamine, cause fetal pulmonary vasodilation during late gestation, demonstrating the capacity for stimulates NOS activity *in vivo*^{9,20,28,56,78}. In contrast with transient vasodilation to many of these agents, responsiveness to exogenous NO or GMP is sustained and present relatively early in gestation². Exogenous (inhaled) NO causes marked and selective pulmonary vasodilation in near-term fetal lambs during ventilation with hypoxic gas⁴⁵. Despite maintaining fetal arterial PO₂ at 20 torr, inhaled NO (5–20 ppm) caused selective and sustained pulmonary vasodilation. The degree of pulmonary vasodilation achieved during exposure to inhaled NO without increasing fetal PO₂ was similar to that achieved by the combined effects of treatment with ventilation and high FiO₂ with or without NO. These findings parallel *in vitro* observations of marked fetal pulmonary artery response to exogenous NO, and led to dose selection for our early clinical studies of inhaled NO in human PPHN (see below). This marked vasodilator response to inhaled NO was also observed in premature lambs^{43,44}.

In summary, in the developing pulmonary circulation, endogenous NO activity influences basal fetal pulmonary vascular tone, can be stimulated by physiologic stimuli (shear stress, ventilation, and increased PO₂) and pharmacologic agonists, contributes to pulmonary vasoreactivity at least as early as 0.7 term in the ovine fetus, and may be enhanced during early postnatal life.

Transitional Lung Circulation:

At birth, the pulmonary circulation undergoes a marked fall in PVR, with blood flow increasing 8–10 fold as pulmonary artery pressure steadily declines^{19,28,29,38}. This rapid decline in PVR is due to vascular dilation, recruitment and distension, which are associated with a rapid structural "reorganization" of small pulmonary arteries. Mechanisms lowering PVR in the early postnatal period include physical factors, such as removal of fetal lung liquid, establishment of an air-liquid interface, rhythmic

distension, increased PO_2 , and shear stress^{1,8,12,19,24,31,41,51,62,86}. Birth-related stimuli act in part by stimulating release of potent vasodilators, such as PG I_2^{52} and NO⁴ (see below), and perhaps by decreasing production of vasoconstrictors, such as leukotrienes or ET-1. Multiple mechanisms have overlapping contributions to the normal decline in PVR at birth, as it appears that no single stimulus is the sole "mediator" of the transition. Physical stimuli can act on the endothelium to selectively release vasoactive substances during the transition. For example, cyclooxygenase blockage attenuates the decrease in PVR at delivery of fetal lambs and goats, which was later shown to be due to inhibition of ventilation-, but not oxygen- induced release of prostacyclin^{26,52,53,85}. In contrast, the NO antagonist, nitro-L-arginine (L-NA), attenuates the decline in PVR by inhibition of both ventilation- and oxygen- stimulated vasodilation in late-gestation fetal lambs^{4,24}. Flow- or shear stress- induced fetal pulmonary vasodilation, an additional birth- related stimulus, also acts through stimulation of prostaglandin¹ and NO release²⁴, but NO antagonists are more effective at blocking this process. These findings suggest that NO contributes to the normal transition of the pulmonary circulation at birth⁴, and that ventilation, increased oxygen, and shear stress are independently capable of stimulating NO activity. It is clear that although its role is essential for postnatal adaptation, NO is not the sole mediator of pulmonary vasodilation during the transition at birth.

To further identify maturation-related changes in NO activity with development, we studied the hemodynamic effects of inhaled NO and NOS inhibition in the premature ovine fetus⁴⁴. NOS inhibition attenuates birth-related stimuli (as in term lambs), and that inhaled NO causes striking vasodilation despite marked prematurity. Although NO activity contributes to the normal decline in PVR at birth in the term and preterm lamb, it remains unknown whether diminished EDNO activity or the inability to release or sustain release of NO at birth contributes to the failure of postnatal adaptation associated with PPHN or HMD^{6,43}.

Role of Nitric Oxide in Experimental Models of PPHN and RDS:

Animal models of pulmonary hypertension have examined the potential role of intrauterine stimuli on perinatal pulmonary vascular structure and function. Studies of the pulmonary vascular effects of chronic hypoxia *in utero* have failed to consistently find histologic evidence of hypertensive changes in fetal or neonatal pulmonary arteries. Although an initial study reported that newborn rats exposed to chronic hypoxia *in utero* have smooth muscle thickening in small pulmonary arteries³⁵, this observation has not been confirmed^{33,65}. Physiologic studies of sheep have suggested that hypoxia caused by placental embolization³⁰, maternal hypotension³⁴ or partial cord compression⁸¹ may alter pulmonary vascular reactivity. Although chronic intrauterine hypoxia does not consistently cause structural or functional abnormalities in the perinatal pulmonary circulation, several studies have clearly shown that chronic intrauterine hypertension alters reactivity and structure, and leads to the failure of postnatal adaptation^{7,55,88}. Levin demonstrated that intrauterine renal artery ligation causes systemic hypertension, which in the setting of a patent ductus arteriosus, transmits high pressure to the pulmonary circulation as well⁵⁴. Increased wall thickness of small pulmonary arteries was found at sacrifice several days later, demonstrating the effects of hypertension on fetal pulmonary vascular structure.

Based on these observations, we developed a model of pulmonary hypertension to examine mechanisms underlying altered physiology and structure in the develop-

ing lung circulation⁷. Inflation of a vascular occluder around the ductus arteriosus in the chronically-instrumented ovine fetus allowed us to study the hemodynamic effects of sustained pulmonary hypertension *in utero* and at birth⁷. Chronic ductus compression elevated pulmonary artery pressure without hypoxemia or sustained increases in pulmonary blood flow. Physiologic studies of fetal vasoreactivity demonstrated the early loss of fetal pulmonary vasodilator response to small increases in PO_2 ⁷. After cesarean-section delivery, PVR remained markedly elevated despite ventilation with 100% oxygen, leading to right-to-left shunting across the ductus arteriosus and foramen ovale and marked hypoxia. At autopsy, striking thickening of small pulmonary arteries and right ventricular hypertrophy were found. Concomitant studies from others further demonstrated that ligation of the ductus arteriosus leads to structural changes and sustained elevation of pulmonary artery pressure, as described above^{63,88}.

To investigate mechanisms which contribute to the failure of PVR to decrease at delivery in this model of experimental PPHN, we examined serial changes in the pulmonary dilator response to vasoactive stimuli in the fetal lamb with pulmonary hypertension⁵⁸. We hypothesized that pulmonary hypertension decreases NO activity, causing altered vasoreactivity and smooth muscle cell proliferation. To test our hypothesis, we compared serial hemodynamic responses to acetylcholine, an endothelium-dependent agonist, and atrial natriuretic peptide (ANP), an endothelium-independent dilator, during chronic ductus compression⁵⁸. We found that acetylcholine-induced fetal pulmonary vasodilation was lost whereas the response to ANP remained intact. After cesarean-section delivery, the vasodilator response to high concentrations of oxygen was markedly attenuated in hypertensive animals, but inhaled NO effectively lowered PVR and improved oxygenation. In contrast, ventilation with 100% oxygen completely lowers PVR in control animals, such that inhaled NO has no additional effect⁵⁸. Greater responsiveness to inhaled NO than high FiO_2 in hypertensive lambs has been previously demonstrated by Zayek^{92,93}.

Overall, these findings suggest that endothelial dysfunction contributes to altered vasoreactivity associated with pulmonary hypertension *in utero*, and during the transition. Mechanisms underlying these findings are unclear, but possibilities include down-regulation of NO synthase; decreased availability of arginine or other substrates; lack of incorporation of NO into EDRF for release from the endothelium; increased NO degradation from superoxide generation; loss of smooth muscle responsiveness due to decreased soluble guanylate cyclase or increased cGMP phosphodiesterase activities; inability to overcome the effects of vasoconstrictor stimuli; or others. Functional changes in smooth muscle may also contribute to altered reactivity in this experimental model¹⁵⁻¹⁷.

Since pulmonary hypertension is associated with severe RDS in human neonates^{6,86}, we investigated the effects of inhaled NO in premature lambs with experimental RDS⁴³. Despite surfactant treatment of premature lambs (115 days; term=150 days), these animals develop progressive hypoxemia and elevations in PVR during prolonged (3 hours) ventilation. In comparison with controls, inhaled NO (20 ppm) effectively lowered PVR and improved gas exchange in lambs with severe experimental RDS. Preliminary data suggests that despite increased pulmonary blood flow with NO therapy, treated animals did not have increased pulmonary edema or permeability, as assessed by wet-to-dry lung weights and a double isotope technique for measuring leak index. These data suggest that inhaled NO may be an effective therapy for premature neonates with severe hypoxemia despite optimal medical management.

Potential Roles for Inhaled NO Therapy of Clinical PPHN and RDS:

Failure of sustained pulmonary vasodilation at birth leads to severe hypoxemia and tissue oxygen deprivation in the human neonate. PPHN is a syndrome which is partly defined as the inability of the pulmonary circulation to achieve the normal decline in PVR during the early perinatal period⁵⁴. As *syndrome*, PPHN is associated with several cardiopulmonary disorders, including asphyxia, sepsis, meconium aspiration syndrome, congenital diaphragmatic hernia, respiratory distress syndrome, lung hypoplasia, and others, or can be "idiopathic" (often referred to as "persistent fetal circulation"). These diverse diseases share common pathophysiologic features, including elevated pulmonary artery pressure leading to right-to-left shunting across the ductus arteriosus or foramen ovale and critical hypoxemia⁸². Additional problems with oxygenation are often due to concomitant intrapulmonary shunting. Many neonates with PPHN recover with standard medical therapies, including hyperoxia, hyperventilation, alkalosis, and pharmacologic drug treatments. However, aggressive hyperventilation may induce lung injury, which may contribute to progressive hypoxemia and increased risk of chronic lung disease. In addition, vasodilator drug therapy can be ineffective due to systemic hypotension (due to the lack of selectivity for the pulmonary circulation), an inability to acutely lower PVR, the inability to sustain vasodilation, or worsening oxygenation due to increased intrapulmonary shunting. Patients who fail conventional therapy often require treatment with extracorporeal membrane oxygenation (ECMO). Although ECMO has improved survival in refractory PPHN, it remains costly, can have severe side effects, and may be associated with significant neurologic sequelae.

The ability of inhaled NO to selectively lower PVR without decreasing systemic vascular resistance was first demonstrated in adults with primary pulmonary hypertension during brief exposure⁷⁰, and by several animal studies in juvenile³² and fetal⁴⁵ and neonatal⁷² sheep. From these early findings, the acute effects of inhaled nitric oxide therapy in newborns with severe PPHN were subsequently studied^{46,47,73}. Two early reports noted acute improvement in oxygenation during inhaled NO treatment of PPHN, using 80 ppm⁷³ or lower doses⁴⁶. The latter report demonstrated that clinical recovery from PPHN could be achieved with continuous low dose NO therapy⁴⁶. Initial study patients consisted of neonates with severe hypoxemia failing to respond to conventional therapies (Fig. 1), including HFOV in many cases. As reported in the initial paper for 6 patients who were treated for ≥ 24 hours, low dose NO (at 6 ppm) caused sustained improvement in oxygenation without causing systemic hypotension or increased methemoglobin levels⁴⁶. During these initial studies, 13/15 consecutive patients with severe PPHN meeting ECMO criteria were successfully treated without needing ECMO therapy^{46,47}.

Thus, inhaled NO can effectively lower PVR (by echocardiographic studies) and improve oxygenation in neonates with severe PPHN, decreasing the need for ECMO therapy. Responsiveness to inhaled NO therapy may be partly dependent on specific diseases associated with PPHN, including congenital diaphragmatic hernia, meconium aspiration syndrome, severe sepsis, idiopathic, and others^{46,47}. In some cases, clinical improvement is better achieved with inhaled NO during high frequency oscillatory ventilation than with either treatment alone. As an adjuvant therapy, inhaled NO provides an additional clinical tool for managing severe PPHN. Multicenter studies are currently underway to determine the overall efficacy, potential toxicity and relative role of inhalational NO therapy in the clinical management of severe PPHN. Data reporting long term follow-up after NICU discharge are needed as well.

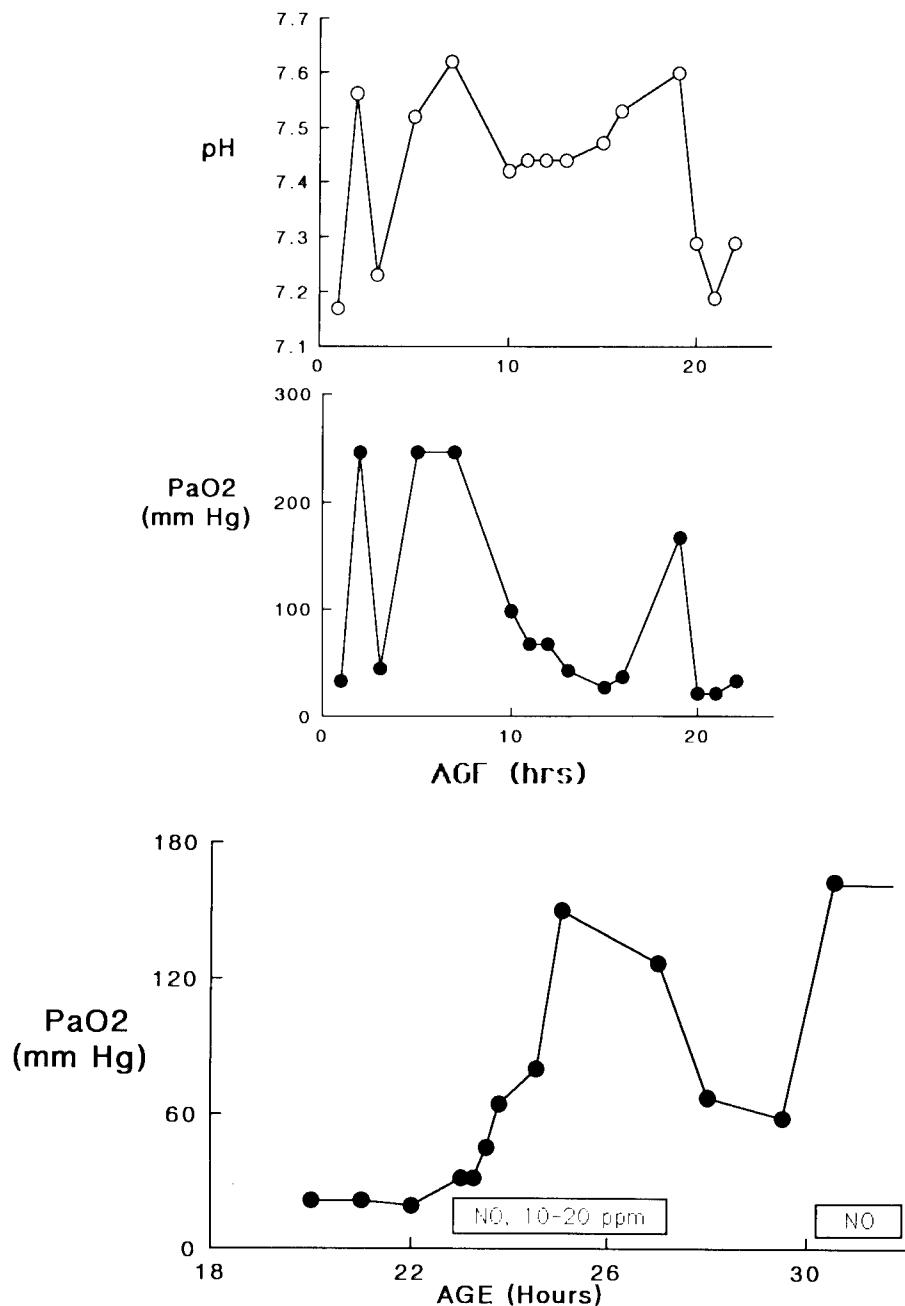


Figure 1 Use of inhaled NO in a neonate with severe PPHN. Although hypocapnic alkalosis due to hyperventilation initially improved oxygenation, PaO_2 decreased to 30 torr with time (Left panel). Initiation of inhaled NO (10–20 ppm) rapidly improved PaO_2 and reversed echocardiographic signs of right-to-left shunting at the ductus arteriosus (right panel). PaO_2 fell after brief withdrawal of NO, but improved with restarting treatment.

Low dose inhaled NO has also been used to treat premature neonate with severe RDS and marked hypoxemia despite aggressive therapy, including repeated doses of surfactant replacement^{22,87}. Pulmonary vasoconstriction can contribute to pulmonary hypertension in the sick premature, leading to similar physiologic abnormalities as observed in PPHN^{6,43}. In addition, low dose inhaled NO may also improve oxygenation by enhancing ventilation-perfusion matching and reducing intrapulmonary shunting. Due to concerns of possible adverse effects (such as prolonged bleeding time and possible extension of intracranial hemorrhage, pulmonary hemorrhage, or increased oxidant stress in a susceptible host), data from clinical trials with long term follow-up is necessary before NO treatment of premature neonates can be recommended.

Potential toxicities from inhaled NO therapy include methemoglobinemia, peroxynitrite formation¹⁴, and increased NO₂ (Fig. 2). Although circulating methemoglobin levels and NO₂ concentrations in the ventilator circuit can be measured, we are unable to measure peroxynitrite formation clinically. Peroxynitrite damages surfactant protein and is cytotoxic *in vitro*³⁶; the potential for similar complications must be studied *in vivo* as well, and remains a major concern. In contrast, however, NO may protect against cellular damage from reactive oxygen species generated from hypoxanthine and xanthine oxidase⁸⁹. In addition, several studies suggest that NO may decrease pulmonary vascular permeability, attenuate lung inflammation by blocking neutrophil adherence to endothelium, and perhaps stimulate epithelial clearance of pulmonary edema⁴⁸⁻⁵⁰. Inhaled NO attenuates the increase in capillary permeability in an animal model of oxidant-induced acute lung injury⁴². Thus, inhaled NO selectively lowers PVR, can redistribute pulmonary blood flow within the lung to improve ventilation-perfusion matching and may reduce lung inflammation and pulmonary edema. Relative risks and benefits of inhaled NO are likely related to dosing strategies, the clinical setting, cautious NO administration, and close monitoring of NO, other nitrogen oxides, and methemoglobin levels.

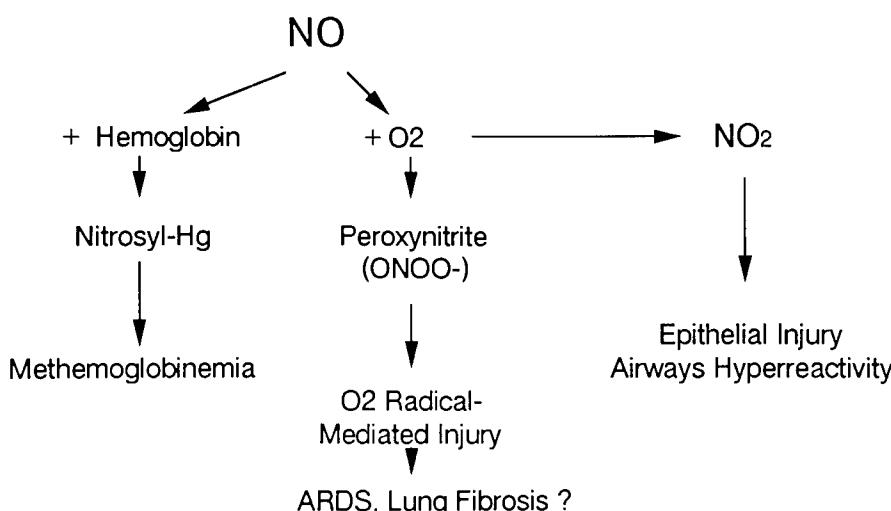


Figure 2 Potential Toxicities of Inhaled NO Therapy.

Conclusions:

Based on experimental studies in the ovine fetus and transitional lamb, NO appears to play a central role in the regulation of perinatal pulmonary vascular tone. Despite high PVR in the normal fetus, NOS is present early during lung development, and basal NOS activity appears to modulate pulmonary vascular tone in the fetus. Stimulation of NO activity contributes significantly to the dramatic fall in PVR at birth, and even the premature fetal lung is markedly responsive to exogenous NO. Furthermore, in experimental models of PPHN and RDS, changes in endogenous NO production or responsiveness contributes to sustained elevations of PVR and hypoxemia, which are at least partly responsive to inhaled NO treatment. Finally, observations from early clinical studies regarding the potential therapeutic roles of inhaled NO in treating hypoxic neonates with PPHN and RDS are promising. Future studies are needed to better define its clinical applications and safety.

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CHAPTER 18

QUANTIFICATION OF HYPOXIA-INDUCED VASCULAR AND EPITHELIAL LEAK

POSSIBLE MECHANISMS

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Introduction

Exposure to high altitude hypoxia may lead to the development of diseases in which edema constitutes one of the main features²². In high altitude pulmonary edema (HAPE), edema develops within the alveoli, resulting from an abnormal transfer of fluid from the pulmonary vessels to the interstitium and to the alveolar space. In high altitude cerebral edema (HACE), associated or not with HAPE, neurological symptoms may result from the compression of brain by fluid leaking from the cerebral vessels. In peripheral edema, observed in the face, ankles and wrists of many subjects ascending to high altitude, interstitial edema sometimes develops without any other sign of malignant mountain sickness. In benign Acute Mountain Sickness (AMS), the mechanisms of symptoms such as headache and nausea are not clearly established but may result from a mild form of cerebral edema. In contrast to the peripheral circulation, the pulmonary circulation is submitted to elevated pressures which are believed to play a major role in the development of HAPE³². The purpose of this paper is to emphasize the possible role of hypoxia-induced increase in vascular and epithelial permeability and its consequences in the development of high-altitude diseases.

Quantification of vascular leak in high altitude hypoxia

Various methods have been used to evaluate vascular leak or development of edema in the lungs or in the periphery. There are few direct methods, based on the measurement of transvascular escape of macromolecules. The most common tracer is radiolabeled albumin (¹²⁵I-albumin)^{9,64,24}. It has been used in monolayers of cultured bovine endothelial cells⁵⁰, in whole animals⁶⁴ and in humans^{9,24}. Indirect methods include the measurement of variations in plasma albumin or hematocrit using a cuff inflated around the arm and the measurement of plasma venous albumin and hematocrit in the exposed and contralateral arm³⁷. Albuminuria is an indirect sign of increased glomerular permeability^{8,24}. A group of methods has been used to explore the

overall alveolar-capillary permeability: intra-alveolar deposition of ^{131}I -albumin¹⁸, pulmonary clearance of inhaled $^{99\text{m}}\text{Tc}$ -diethylenetriaminepentacetate (DTPA)⁴⁰. Lung weight gain⁴ and increase in lung lymph flow^{74,7,41} have been extensively used as markers of lung vascular permeability. Peripheral vascular permeability as well as blood flow can be explored by venous occlusion plethysmography using a mercury gauge around the calf, after inflating a cuff around the thigh at 50 mmHg¹⁴ (Fig. 1). The mean capillary permeability coefficient calculated from this method increased by 34 % after 24 hours at 4,350m and was well correlated to the AMS score (Fig. 2). Whole body weight gain is an overall indirect method to evaluate water retention; as vascular compliance is limited, the extra- water retained is supposed to be mainly located in interstitial space^{22,53}. Recently, an ultrasound technique was developed to measure skin thickness variations, as an indirect parameter of cutaneous interstitial edema [20]. Finally, lung radiography, brain scan, muscle echography, and nuclear magnetic resonance can be used as indirect indices of water accumulation in tissues.

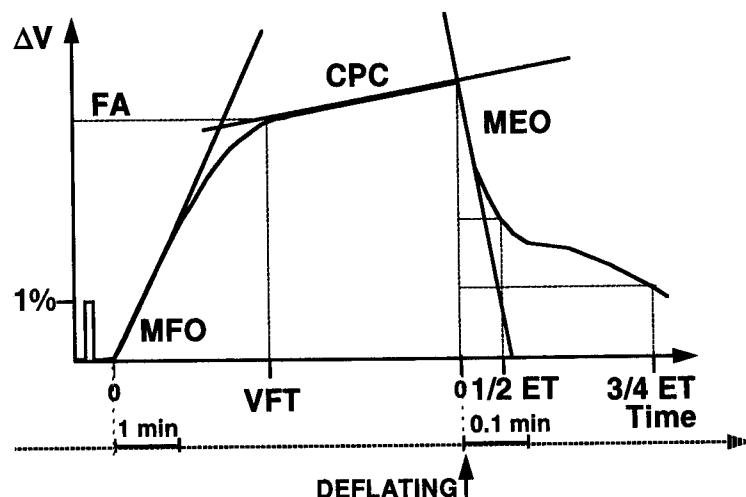
The mechanisms of vascular leak can be derived from Starling's equation (Fig. 3):

$$Q_f = K_f \cdot [(P_{pl} - P_i) - \sigma (P_{pl} - P_{li})]$$

Increase in net fluid flux can be due to :

- 1). Increased hydrostatic pressure : P_{pl} (ex.: left ventricular failure)
- 2). Decreased oncotic pressure : P_{li} (ex.: renal failure, hypoalbuminemia)
- 3). Increased filtration coefficient : K_f . (ex.: toxic pulmonary edema, poisoning).

Plethysmographic recording



CPC : capillary permeability coefficient

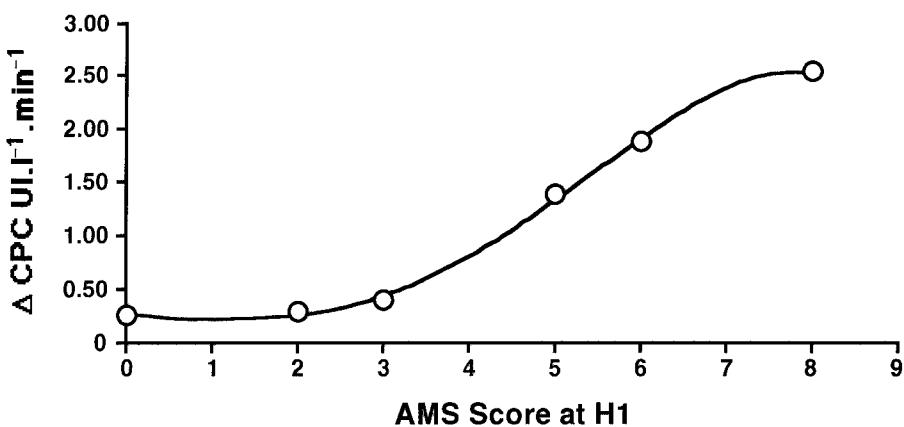
VFT : venous filling time

MFO : maximum filling output

MEO : maximum emptying output

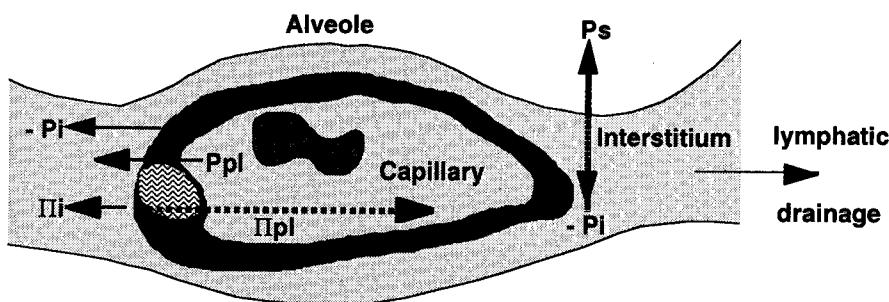
Figure 1 Plethysmographic recording of the lower limb using a mercury gauge transducer at 4,350m. A cuff is inflated around the thigh at a pressure of 50 mmHg. The gauge is placed around the calf and the variations of its resistance are in proportion with the volume of the calf. CPC: capillary permeability coefficient, MFO: maximum filling output, FA: filling amplitude; VFT: venous filling time; MEO: maximum emptying output; 1/2 ET and 3/4 ET : 1/2 and 3/4 emptying time. CPC increased at high altitude. The observed decrease in VFT, FA, MEO and 1/2 ET could be explained by a decrease in venous compliance.

Relation between CPC variations and AMS Score at high altitude



CPC : capillary permeability coefficient
 H1 : 24 hours at 4350 m

Figure 2 Relation between AMS score (Lake Louise Consensus) and Capillary Permeability Coefficient evaluated by mercury gauge plethysmography, after 24 hours at 4,350m.



$$\text{Starling equation : } \dot{Q}_f = K_f \cdot [(P_{pl} - \dot{P}_i) - \sigma(\Pi_{pl} - \Pi_{li})]$$

where : \dot{Q} : net transvascular fluid flux \dot{P}_i : plasma
 K_f : fluid filtration coefficient i : interstitium
 P : hydrostatic pressure s : surface
 Π : protein osmotic pressure
 σ : plasma protein reflection coefficient

Figure 3 Forces involved in transfer of fluids in the lung

Experimental evidence of hypoxia-induced increase in capillary permeability was first demonstrated by Landis in 1932³⁷. Thereafter, studies gave conflicting results. Some authors showed experimental evidence of increased lung vascular permeabil-

ity^{42,74,27,12,78,32,48,44}. Other studies failed to demonstrate any increase in vascular permeability or edema formation in the lung^{26,47,18,15,43,66,7,38,28}. Contradictions observed may be due to differences in species, experimental design or sensitivity of the method used. Pigs and rats, and especially some strains of Sprague Dawley rats seem more sensitive to pulmonary edema than sheep or dogs^{10,57}. However, Viswanathan found comparable incidence of HAPE in dogs and rats and a higher incidence in mice⁷⁰. Experimental design seems determinant, particularly the duration and intensity of hypoxia. Studies where exposure to hypoxia is short (a few minutes or hours) are less likely to cause pulmonary edema³⁸. When exposure to hypobaria is abrupt, mechanical injuries are more likely to occur⁴⁵. Studies where duration of exposure is 24 to 48 hours are more likely to mimic what happens in humans exposed to high altitude and developing pulmonary edema⁶⁴. The sensitivity and specificity of the techniques used to evaluate the vascular leak can also be determinant. Some methods specifically explore vascular permeability, others give indirect indices of vascular leak, such as lung water or lymph flow, some explore both endothelial and epithelial resistance to fluid flux.

Mechanisms of increased filtration coefficient in HAPE.

Increase in capillary permeability can be induced either by mechanical or humoral processes. Mechanical processes include widened opening of intercellular junctions ("stretched pore" hypothesis)^{59,62} or disruptions of endothelium/epithelium ("stress failure" hypothesis)⁷⁶. In these processes, increased hydrostatic transvascular pressures are mainly responsible for the leak. However, metabolic-induced modification in the shape of the cells can be responsible for the widening of intercellular junctions⁶⁴. Humoral processes include the release of mediators by macrophages, neutrophils, platelets, endothelial cells or epithelial cells. However, the nature of the mediator(s) is still debated. Histamine^{3,21} and bradykinin⁴⁸ have been incriminated. The hypothesis of an hypoxia-induced activation of phospholipase A2⁷³ led to the study of metabolites of arachidonic acid as mediators of altitude edema (leucotrienes, prostaglandins, thromboxane). Some of them were found elevated in the alveolar fluid of HAPE subjects⁵⁸, in a model of mechanical lung injury⁶⁹ and in plasma at high altitude⁵⁴. Atrial natriuretic peptide release at high altitude is controversial. It could be released in response to elevated pulmonary arterial or right atrial pressures^{5,11,34} and induce an increase in capillary permeability⁷⁵.

Oxygen radicals have been shown to induce vascular injury after reoxygenation and may be involved in hypoxia-induced lipid peroxidation in rat lungs⁶⁷. Cytokines (TNF, IL1, IL6) and nitric oxide (NO) could also play a role but their specific action on capillary permeability remains to be established. Adhesion molecules, present on the surface of endothelial cells and neutrophils, have recently been shown to participate in the inflammatory process¹⁷. Endothelial leukocyte adhesion molecule-1 (ELAM-1), Intercellular adhesion molecule-1 (ICAM-1) and Vascular cell adhesion molecule-1 (VCAM-1) are some of the molecules that can be found in plasma and serve as markers of endothelium or neutrophils activation. Mediators such as thrombin, histamine, and cytokines (TNF, IL1) can activate adhesion molecules, via a *de novo* protein synthesis. Lung vascular injury could be prevented by pre-treatment with anti-adhesion molecule substances.

Some experimental evidence and human studies show an hypoxia-induced increase in plasma endothelin. In a study performed on 10 subjects, plasma endothelin

was increased by 51% after one week of exposure to 6,542m. However, moderate or severe exercise failed to stimulate endothelin release and the exercise-induced increase in plasma renin activity was inversely related to plasma endothelin^{55,13}. In a study at the Observatoire Vallot, endothelin increased by 82% after 5 days at 4,350m; in parallel to the endothelin release, adhesion molecules (ELAM-1) were found elevated in plasma (Table 1)¹³.

Table 1. Endothelin and adhesion molecules at high-altitude

	ET pg/ml	ELAM-1 ng/ml	ICAM-1 ng/ml
6,542 m (n=10)			
Normoxia	5.7±0.9	/	/
Hypoxia, 1 week	8.6±2.4***	/	/
Hypoxia, 3 weeks	7.7±1.4**	/	/
4,350 m (n=5)			
Normoxia	3.9±1.3	26±13	215±45
Hypoxia, 5 days	7.1±1.4*	37±17*	239±21

H vs N : *, p<0.05, **, p<0.01, ***, p<0.001

ET: plasma endothelin; Endothelial leucocyte adhesion molecule -1 (ELAM-1), Intercellular adhesion molecule-1 (ICAM-1).

The effects of hypoxia on endothelial cell permeability was investigated by Ogawa *et al.*⁵⁰. Cultured bovine endothelium cells in monolayer were exposed to $PO_2 \geq 14$ mmHg. The permeability to macromolecules was accelerated in a time-dependent and dose-dependent manner. A significant increase was shown after 24 to 48 h, with a maximum at 72 h. It was reversible within 48 h after reoxygenation. These variations were associated with morphological alterations with larger cells and the presence of intercellular gaps. Thrombomodulin activity (a cell surface anticoagulant cofactor) was inhibited by hypoxia and reversed by reoxygenation.

The effects of hypoxia on pulmonary vascular leak were investigated in rats by Stelzner *et al.*⁶⁴. Rats were exposed for 1 to 48 hours at 450 mmHg. The authors showed an increase in transvascular protein escape, inhibited by glucocorticoid pre-treatment, augmented by adrenalectomy, and independent of pulmonary arterial pressure. This phenomenon was not seen for short exposures (1 to 13 h), was similar in normobaric or hypobaric hypoxia. It was associated with increased lung water and perivascular edema cuffs on histological study.

Relevance

How are these studies relevant with respect to the pathophysiology of HAPE? Some historical studies on hypoxia-induced alterations in lung morphology or function and capillary permeability are worth citing to show how the diagnosis of hypoxia-induced pulmonary edema found its way in the beginning of the century. As early as in 1922, Harrop established a relationship between an alteration in lung diffusion capacity and the symptoms of AMS²⁵. In 1925, Bayeux exposed guinea-pigs and rabbits to hypobaria (hypobaric chamber and Observatoire Vallot, 4350m). He found

"hypertrophy of alveolar walls - accumulation of alveolar cells, bathing in serosity which reduces the volume of alveoli and compresses the peri-alveolar capillaries, inducing a resistance to circulatory flow, pulmonary hyperemia and right heart dilatation"⁶. In 1939, Piéry studied geese and rabbits at the Jungfraujoch, 3457m and found "alveoli with hemorrhages, a great congestion of lung capillaries, congestive alveolar walls, normal heart"⁵¹. In 1942, Warren and Drinker showed an hypoxia-induced increase in pulmonary lymph flow in anesthetized dogs⁷⁴.

As early as 1957, from clinical observations made between 1952 and 1955, Bardalez and Lizarraga^{23,39} offered a precise hypothesis for HAPE ("edema agudo por soroche grave"): 1) high pulmonary capillary pressure; 2) increased capillary permeability; 3) decreased blood osmotic pressure; 4) increased sympathetic stimulation, inducing a displacement of blood from periphery to the lungs; 5) increased secretion of epinephrine, corticosteroids and local humoral agents such as histamine. Heart failure and pulmonary infection were discarded as possible causative factors by the authors who described HAPE as a "neurohemodynamic" edema.

In the history of concepts of the physiopathology of HAPE, the hypothesis of increased vascular permeability had some success (Fig. 4). The increased pulmonary arterial pressure was considered to be the main feature in mechanisms of HAPE^{29,31}. From post-mortem studies, a fibrin-rich alveolar edema led to the hypothesis of capillary damage⁴⁶ or increased capillary permeability¹. Indian authors attribute to increased capillary permeability a part of the responsibility for acute mountain sickness and its edematous manifestations^{60,61,71}. Whayne and Severinghaus located the leak in the small arterioles⁷⁸. Subclinical interstitial pulmonary edema is a common finding in newcomers to high altitude, without HAPE^{36,19,65,33}.

Evolution of the concepts in the pathophysiology of HAPE

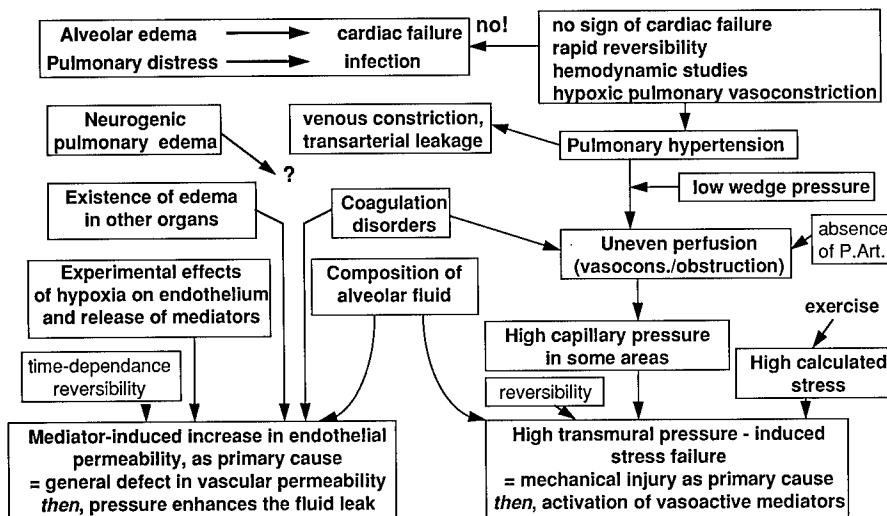


Figure 4 Evolution of the concepts in the pathophysiology of HAPE

In 1972, Fishman pointed out the role of the alveolar rather than endothelial barrier, and the role of the lymphatic drainage to limit the interstitial edema¹⁶ and in 1978, Staub hypothesized that shear stress-induced alteration of endothelium leads to a leak of fluid into the interstitium⁶². Increased permeability was proposed as a common mechanism for all altitude-induced edema²².

However, the specificity of high altitude peripheral edema has been questioned since it was also observed in subjects trekking at low altitude⁷⁹. Neutrophils are incriminated in the release of mediators inducing a "high-permeability type" of pulmonary edema⁶³. Proteinuria is suggestive of a generally increased permeability⁸. The "pore-stretching" hypothesis is suggested by the rapid reversibility of the edema⁵⁶. Leukotrienes, prostaglandins, dopamine may play a role in the onset of edema³⁰. In 1986, data on the composition of alveolar fluid were in favor of a "high-permeability" edema^{58,23}. As Kobayashi wrote in 1987, "albuminuria, pulmonary edema, papilledema, cerebral edema all frequently observed in HAPE, suggest the existence of a body-wide defect in vascular permeability". The conclusion drawn by Oelz in 1989 for the pathophysiology of HAPE is "permeability edema in which hypoxic pulmonary hypertension is a crucial factor by enhancing the flow of liquid across the damaged endothelial barrier"⁴⁹. The concept of uneven vasoconstriction is questioned by the findings of Vock *et al.* who showed the evolution of X-ray images from "patchy" to "homogeneous" and clinical findings suggesting that structural abnormalities are not involved in the pathogenesis⁷². In the "stress failure" hypothesis^{76,77,68}, high capillary hydrostatic pressure breaks endothelial and/or epithelial barriers, exposes basement membrane, and activates the release of vasoactive mediators. The mechanical damage is the primary cause, and permeability is increased by the release of mediators.

Is a vascular or an epithelial leak responsible for hypoxia-induced alveolar edema? In fact, it is important to consider that vascular permeability to fluid is a normal physiological process. Moreover, a large number of individuals develop lung interstitial edema at high altitude, without HAPE. Lung interstitial edema occurs with a few mmHg increase in vascular hydrostatic pressure. Compliance of lung vessels and interstitium is great. Only a 5 to 8% increase in lung weight will be associated with peribronchovascular edema. As much as a 35% increase in lung weight is necessary to be associated with alveolar edema. Epithelial permeability is 10 times lower than endothelial permeability. Thus, the epithelium is a much stronger barrier than the endothelium. A question arises: Is alveolar epithelium and not capillary endothelium the limiting factor for the development of alveolar edema?

Recent studies indicate that certain pneumocyte functions may be altered in hypoxia⁵². Sodium transport by alveolar epithelium represents an important mechanism for airspace fluid clearance in acute lung injury. We asked whether hypoxia affects Na,K-ATPase activity in alveolar epithelial cells (AEC). SV40 virus-transformed rat type II AEC were exposed to either hypoxic (5% O₂) (H) or normoxic (21% O₂) (N) for increasing times up to 48 h in the absence or presence of 10⁻⁵ M nifedipine. Na,K-ATPase activity was determined using ouabain-sensitive ⁸⁶rubidium influx (OsRb). Exposure to hypoxia for at least 12 h induced a time-dependent decrease of OsRb (101 ± 9.7 in H vs 218 ± 14 nmol/10min/mgprot in N after 24h; p<0.001). Incubation of N cells with supernatant of H cells resulted in a 45% decrease of OsRb within 1 h. Nifedipine prevented the hypoxia-induced decrease in OsRb (Fig. 5). These results indicate that: 1) hypoxia induces a time-dependent decrease of Na,K-ATPase

Effect of Nifedipine on Hypoxia - Induced decrease of Na,K - ATPase Activity

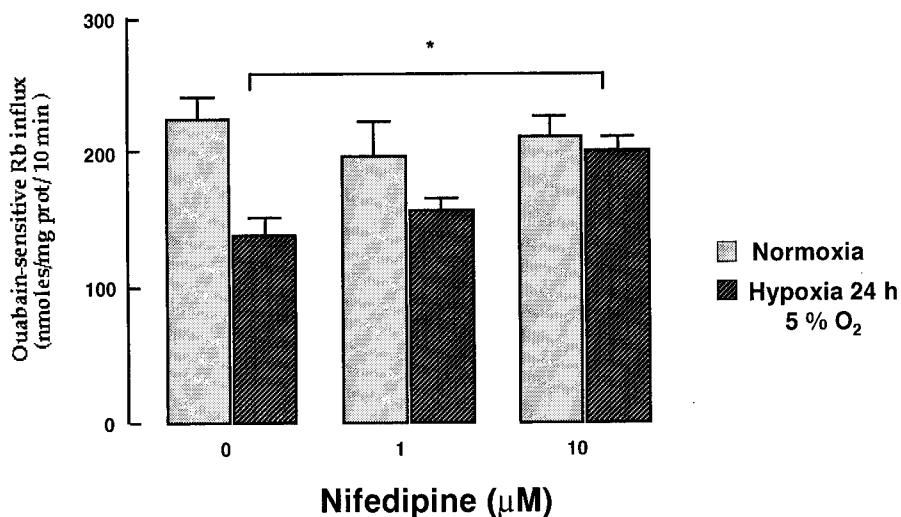


Figure 5 Hypoxia-induced inhibition of Na, K ATPase in cultured PII pneumocytes. Protective effect of nifedipine.

activity in alveolar type II cells, 2) this effect is most likely due to the release of a soluble factor and is prevented by nifedipine. Autocrine alteration of AEC Na,K-ATPase activity during hypoxia may reduce airspace fluid clearance and contribute to the formation and/or maintainance of alveolar edema.

Conclusion

The hypoxic challenge triggers important modifications in the vascular system both in the pulmonary and the peripheral circulations. Increase in interstitial or tissue edema is a main feature of the altitude-induced pathological manifestations. An exacerbation of the permeability properties of the endothelium could be directly mediated by hypoxia or by substances locally released by endothelial cells or neutrophils. In the pulmonary circulation, another factor interferes with the increased vascular permeability, namely hypoxic arteriolar vasoconstriction and pulmonary hypertension. Interstitial edema has only limited functional consequences. When edema develops within the alveoli or in the brain, severe consequences may appear. Hypoxia-induced alterations of the alveolar epithelial cells could be one of the mechanisms by which lung interstitial edema turns into alveolar edema.

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CHAPTER 19

TRANSCAPILLARY ESCAPE RATE OF ALBUMIN AT HIGH ALTITUDE

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Abstract

High altitude pulmonary edema is characterised by an increase in the pulmonary artery pressure while an increase in systemic vascular permeability as a pathogenetic factor is still under debate. Therefore, we investigated the effects of high altitude exposure on the transcapillary escape rate of albumin, a reflection of the vascular permeability, by injecting 5 µCi [¹²⁵I]albumin in 33 healthy subjects at 550m and after active ascent at 4559m. Condensates of exhaled air from 24 subjects were collected by a new system using a cool trap. Four subjects developed high altitude pulmonary edema and 14 acute mountain sickness. The transcapillary escape rate of albumin was 6.4±2.1 %/h at low and 6.3±1.8 %/h (p>0.05) at high altitude. There was no radioactivity, albumin or interleukin-8 detectable in the condensates of exhaled air. The present findings do not support the hypothesis that exposure to high altitude causes a primary systemic vascular leak detectable by measuring the transcapillary escape rate of [¹²⁵I]albumin even in subjects suffering from high altitude pulmonary edema.

Introduction

High altitude pulmonary edema (HAPE) is characterised by an increase in pulmonary artery pressure and normal left ventricular function reflected by a normal wedge pressure¹³. Hypoxia-induced pulmonary artery hypertension was shown to be considerably enhanced in HAPE-susceptible subjects^{18,19,25}. Furthermore, it was shown in recent studies that exaggerated hypoxic pulmonary vasoconstriction precedes edema formation⁴, and that nifedipine is effective for both treatment²¹ and prevention⁴ of HAPE. The beneficial effects of nifedipine are likely due to lowering pulmonary artery pressure. However, whereas pulmonary artery hypertension is a well established pathogenetic factor, high protein concentrations found in broncho-alveolar lavage²³ of subjects suffering from HAPE demonstrate that an increase in vascular

permeability may be an additional factor contributing to HAPE. Moreover, there is indirect evidence suggesting an increased capillary permeability in subjects with acute mountain sickness (AMS) and even in those exposed to high altitude without symptoms^{7,14}.

Coates *et al.*⁸ investigated the influence of hypobaric hypoxia (4300m) on the transcapillary escape rate (TER) of albumin in a pressure chamber. They found an increase of TER from 6.2 %/h to 7.6%/h at high altitude which was not significant. In contrast, Hansen *et al.*¹⁴ recently demonstrated a significant albeit small increase in TER when healthy subjects were flown from sea level to 4350m. The mechanism of the postulated increase in vascular permeability was not further assessed in their study. From investigations in patients at low altitude we know, however, that hypoxia itself probably does not cause an increase in TER of albumin. Henriksen *et al.*¹⁵ investigated the TER of albumin in patients suffering from chronic obstructive pulmonary disease associated with pronounced hypoxemia and have not found an increase in TER when compared to healthy control subjects. This finding was supported by animal studies, in which edema formation was not altered by gradually lowering the inspiratory oxygen concentration to zero¹⁶.

There are various other factors that might influence vascular permeability at high altitude which have so far not been investigated. Any inflammatory reaction can cause increased vascular permeability². Thus, Fleck *et al.*¹² studied the mechanisms of hypoalbuminemia in patients undergoing open heart surgery and found a substantial increase in TER of albumin. The responsible pathogenetic factor causing increased vascular permeability in inflammatory conditions might be the secretion of cytokines. Thus, interleukin (IL)-2 was shown to increase the permeability of pulmonary arterial endothelial cells *in vitro*¹⁰. In a recent study, we have shown that TER of albumin increased up to 50% in patients treated with IL-2 for malignant melanoma³.

In the present study we therefore investigated whether high altitude exposure associated with physical exercise would induce an increase in vascular permeability and whether this would be accompanied by an increase in the concentrations of proinflammatory cytokines in the blood and the condensates of exhaled air.

Methods

Thirty-three patients were investigated after an overnight fast at 550m and 2 to 3 weeks later after active ascent to 4559m (Capanna Regina Margherita) during studies performed in 1993 and 1994. TER of albumin was measured by injecting 5 µCi [¹²⁵I]albumin intravenously¹⁻³. Blood samples were drawn from a cubital vein of the opposite arm at 10 min intervals up to 60 min and the plasma samples were immediately frozen in liquid nitrogen. Later, 2 ml of plasma were counted on a gamma-counter and the counts were plotted against time. TER was calculated as described previously (2,3) according to the formula:

$$\text{TER } (\%/\text{h}) = (\alpha \times 60 \times 100)/y$$

where α is the slope of the linear regression and y the intercept of the linear regression line on the y -axis.

Breath samples for analysis of radioactivity, albumin and interleukin-8 were collected according to the method of Scheideler *et al.*²² in all 24 subjects of the study performed in 1993. In brief, the subjects had to breathe for about 20 to 30 min through a system shown in Figure 1. The exhaled air (exactly 300 L) of the subjects was

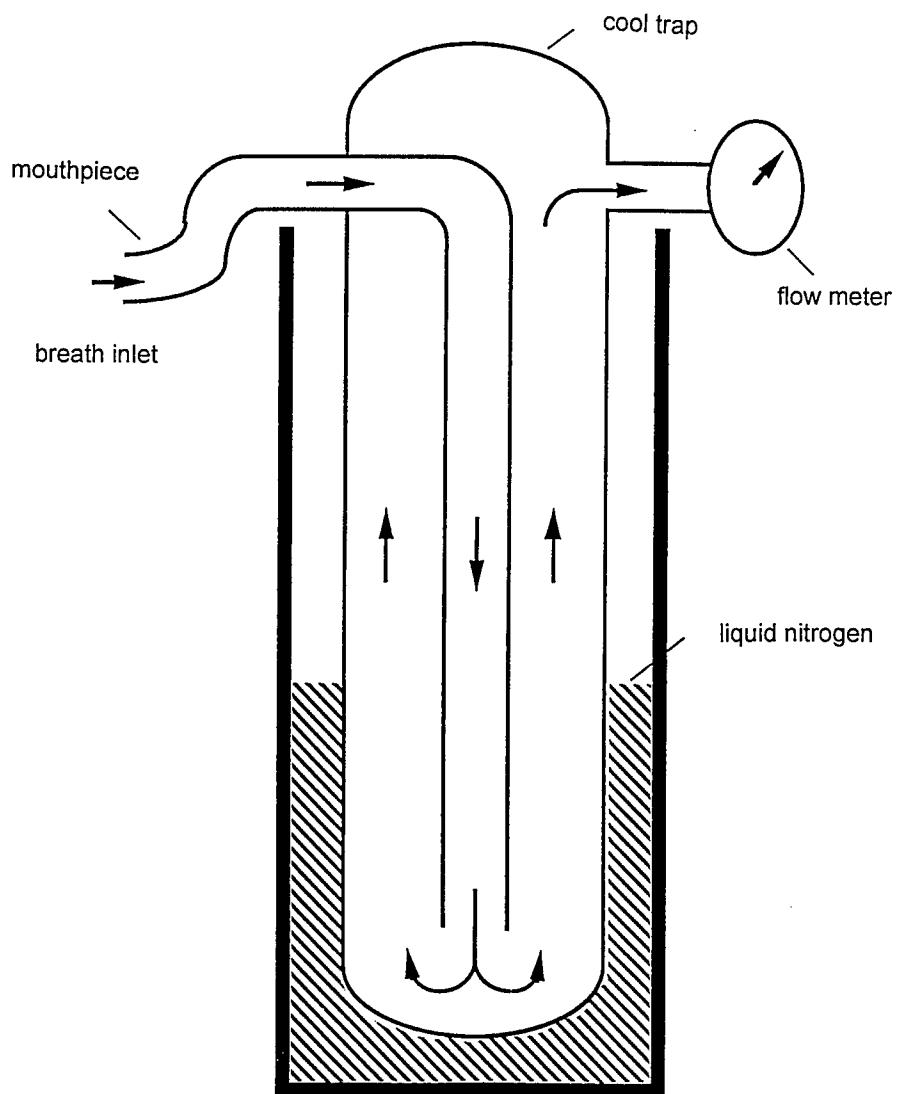


Figure 1 System for the detection of non-volatile macromolecules in breath. The subjects were exhaling through the mouthpiece for about 20 to 30 min, i.e. 300 L of air. The air passed through the cool trap filled with liquid nitrogen; later, the frozen condensates of exhaled air were defrosted and sampled in assay tubes for future analysis.

conducted through a cool trap of liquid nitrogen. Potentially contaminating saliva was diverted by the S-shaped mouthpiece. The frozen condensates of exhaled air were defrosted and collected in assay tubes for future analyses. The condensates were analysed for radioactivity, albumin (by ELISA technique, Immunozyme rec Albumin^R Immuno GmbH, Heidelberg, Germany) and interleukin-8⁶. Plasma IL-8⁶ and IL-2 (Quantikine, RD Research and Diagnostics System, Minneapolis) concentrations were measured by ELISA technique.

The diagnosis of HAPE was based on radiographic findings as described previously^{4,24}. AMS was defined by a Lake-Louise score ≥ 5 (self-report and clinical assessment) or ≥ 4 (self-assessment only) at the time of the examination and/or within the following 24 h at high altitude, as the values of these scores are comparable to an AMS-C score of $\geq 0.70^5$.

Results

Four of the 33 subjects had radiographic evidence of HAPE, and 14 suffered from AMS at the time of the examination or within the following 24 h. In Figure 2 the values of TER of albumin are shown at low (550m) and high altitude (4559m). We have found a relatively wide scatter of TER as described previously¹⁻³. The TER was $6.4 \pm 2.1\%/\text{h}$ at low and $6.3 \pm 1.8\%/\text{h}$ ($p > 0.05$) at high altitude. The values of all subjects are summarised in Table 1.

In 5 of the 24 subjects in whom breath samples were collected, albumin was detected in the condensates of exhaled air with concentrations below 10 $\mu\text{g}/\text{mL}$ (detection limit 0.8 $\mu\text{g}/\text{mL}$), and in 2 subjects with concentrations of 32 and 120 $\mu\text{g}/\text{mL}$. The distribution of the positive results was random (Table 2). Contamination with

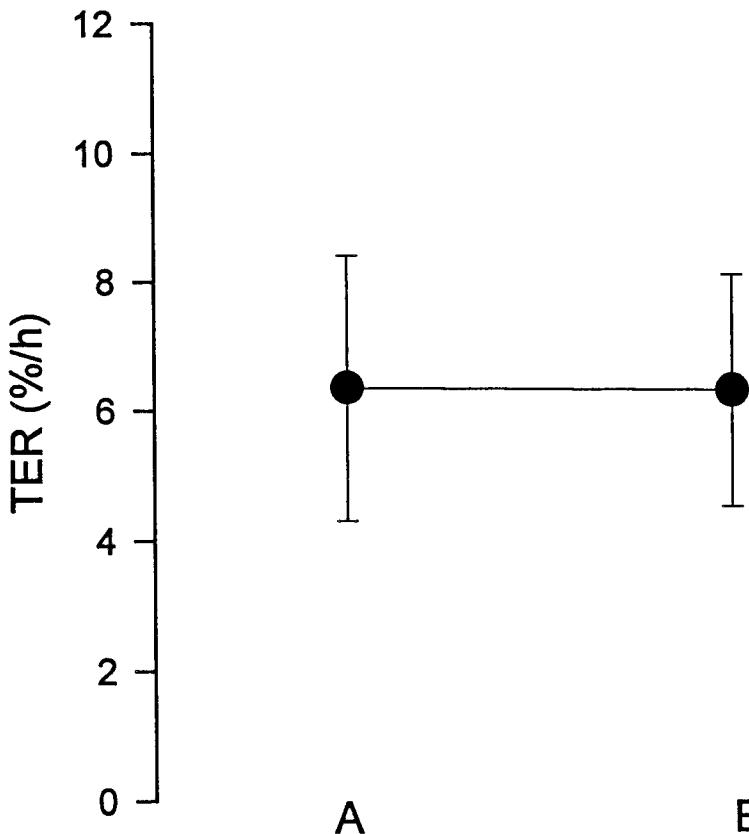


Figure 2 Transcapillary escape rate (TER) of albumin at 500m (A) and at 4559m (B). There was no difference in the values of TER between the two time points ($p > 0.05$).

saliva might have occurred in some subjects with detectable albumin, in particular in the 2 subjects with the highest concentrations measured at high altitude. It is noteworthy that no condensate of exhaled air of the subjects with beginning HAPE was positive for albumin. There was also no interleukin-8 detectable in the condensates. IL-2 and IL-8 plasma concentrations were not elevated at both time points.

Table 1

Transcapillary escape rates in %/h at 550m (A) and at 4559m (B) in subjects without symptoms (normal), with acute mountain sickness (AMS), and with high altitude pulmonary edema (HAPE). There were no significant changes between A and B in all 3 groups by Wilcoxon signed rank test.

Subject	Normal (n=15)		AMS (n=14)		HAPE (n=4)	
	A	B	A	B	A	B
Mean	6.6	6.4	6.6	6.3	4.6	5.7
Sd	2.1	1.9	1.9	1.8	1.9	1.9

Table 2

Distribution of albumin-positive condensates of exhaled air at 550m (A) and at 4559m (B). There were no significant changes by Chi-square test.

Normal (n=10)		AMS (n=10)		HAPE (n=4)	
A	B	A	B	A	B
1	1	1	4	0	0

Discussion

In contrast to earlier studies^{8,14}, we have not found an increase in vascular permeability at high altitude measured by injecting radiolabelled albumin. Fourteen subjects (of the 33) suffered from AMS and 4 had radiographic evidence of HAPE at the time of the measurement of TER or within the next 24 h. Hansen *et al.*¹⁴ found relatively low values of TER at sea level when compared with our base line values, although some of the present subjects also had TER values of 4 %/h and lower. The increase of TER from 4.8 %/h to >6.7 %/h at high altitude in their study is relatively modest and might be confounded by a lack of statistical power. We have calculated the statistical power for the TER data in our two studies (i.e. the 1993 and 1994 study) with 33 subjects, and have found the power to be high: assuming an increase of TER by 2.5 %/h (e.g. from 5 %/h to 7.5 %/h) at high altitude and an α -error of 5 percent, the β -error would be less than 5 percent with 33 subjects. In contrast, Hansen *et al.*¹⁴ have investigated TER in only 9 (out of 12) subjects and might erroneously have found an increase in TER because of the relatively small number of subjects. More-

over, they have compared subjects treated with either placebo or isradipine—a calcium-channel blocker. Four of the 6 subjects who showed an increase in TER were under treatment with isradipine whereas only 2 of the subjects with placebo had an increase in TER¹⁴. This might suggest that isradipine itself had some influence on TER. Calcium-channel blockers are known to induce edema formation¹¹ which is probably caused by an increase in vascular permeability and might therefore be the basis of the increase in TER in their study.

Although the permeability for tracer albumin or sorbitol increased when endothelial monolayers were exposed to low oxygen concentrations²⁰, hypoxia had no effect on albumin permeability in both experimental animals¹⁶ and humans¹⁵. Other factors such as inflammatory mediators are involved in the pathogenesis of an increase in TER. IL-2 is among the most important cytokines which has the potential to increase vascular permeability in both cell culture systems, i.e. pulmonary endothelial cells¹⁰, and in humans³. In line with those findings both TER and plasma IL-2 concentrations were not increased in the present subjects at high altitude. Even the four subjects suffering from HAPE showed no increase in IL-2 plasma concentrations.

Since plasma levels of inflammatory mediators might not reflect the local concentrations we have tried to examine the condensates of exhaled air which might indicate a potentially serious capillary leak by increased albumin concentrations and/or inflammatory mediators. Recently, Scheideler *et al.*²² described a new non-invasive system for the detection of non-volatile macromolecules in the breath. This is based on the assumption that non-volatile components in exhaled air are transported as aerosols representing all lung spaces connected with the conducting airways²². Scheideler *et al.* were able to detect several inflammatory mediators, e.g. IL-1 β and soluble IL-2 receptor protein, in patients with pulmonary disease and in control subjects with recent onset of airway infection²². However, we have not found radioactivity or albumin in the condensates of exhaled air.

Interleukin-8 was demonstrated to be a potential marker for the development of the acute respiratory distress syndrome⁹ a condition which bears some resemblance to HAPE. We therefore hypothesised that IL-8 might be detectable in the patients suffering from HAPE. However, IL-8 was neither found in the condensate of exhaled air nor the plasma which suggests that no primary inflammatory reaction occurred at high altitude. Our findings are in contrast to the results obtained by Schoene *et al.*²³ a few years ago. They performed broncho-alveolar lavage in climbers with HAPE at an altitude of 4400m²³ and found high molecular weight proteins, cellular components (e.g. erythrocytes and alveolar macrophages) and inflammatory mediators such as leukotriene B₄ in the lavage fluids. However, in contrast to our subjects who had beginning HAPE, their subjects had severe forms of HAPE lasting for many hours or a few days, which might suggest that the inflammatory reaction was of secondary character and not the pathogenetic factor leading to HAPE.

In summary, we have not found an increase in the systemic vascular permeability in a relatively large number of subjects exposed to high altitude, although some of them suffered from HAPE and half of them had AMS. Moreover, condensate of exhaled air analysis did not indicate that the pulmonary vascular permeability increased to an extent which might allow albumin or IL-8 to escape into the alveolar space.

In conclusion, exposure to high altitude does not cause a substantial primary capillary leak syndrome, and the reported increase of the pulmonary arterial pressure^{4,17,21} remains the most important pathogenetic factor for the development of HAPE. A

minimal capillary leak, however, cannot be definitively excluded by the present study because both methods used might not be sensitive enough. Moreover, the statistical power was not big enough to exclude an increase of TER in the 4 subjects with HAPE. After all, three of the 4 subjects showed a moderate increase in TER and thus a larger number of subjects developing HAPE should be investigated in the future.

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CHAPTER 20

CYTOKINES AND ADHESION MOLECULES IN HIGH ALTITUDE PULMONARY EDEMA

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Introduction

The pathogenesis of high altitude pulmonary edema (HAPE) is still not completely defined. There is considerable evidence that elevated pulmonary vascular pressure plays a significant role in the development of HAPE^{3,17,18,21-23}. Yet it is uncertain whether elevated pulmonary vascular pressure alone can account for the nature of the pulmonary edema fluid seen in HAPE. We and other investigators^{4,5,17,32,33} have questioned whether an inflammatory-mediated mechanism is involved early in the development of HAPE. Postmortem findings of lung tissue from HAPE victims have demonstrated fibrin thrombi, hyaline membranes and neutrophil sequestration^{1,10,28}. Furthermore lung lavage fluid from climbers acutely suffering from HAPE were found to have a nearly five-fold greater white cell count than healthy climbers^{16,32,33}. Although alveolar macrophages (AM) were the predominant cell type (67%), the polymorphonuclear leukocyte count was also markedly high. In addition, elevated levels of thromboxane B₄ (TxB₄), leukotriene B₄, and complement C5a were found. Thromboxane B₄ is a potent pulmonary vasoconstrictor that mediates platelet and neutrophil aggregation and is released from platelets and alveolar macrophages following stimulation by the pro-inflammatory cytokines such as tumor necrosis factor- α (TNF- α)²⁰. Leukotriene B₄, also produced by activated AM, is a potent neutrophil chemotactic agent²⁷.

Although these data strongly suggest an inflammatory-mediated mechanism in HAPE, it is difficult to determine if pro-inflammatory mediators are involved in the initiation of the lung injury or whether they are activated following mechanical injury to the pulmonary vascular bed. In this paper I will present data from our laboratory and others suggesting that pro-inflammatory cytokines and endothelial adhesion molecules may have an early role in the pathogenesis of HAPE.

Pro-inflammatory Cytokines and E-selectin in HAPE-Susceptible Subjects

Recently a family of cell surface adhesion glycoproteins called selectins has been identified that is expressed on endothelial cell surfaces, leukocytes and platelets in

response to a variety of stimuli and inflammatory mediators^{6-8,37} (Table 1). Of particular interest is endothelial leukocyte adhesion molecule (E-selectin) which is expressed only on activated endothelial cells induced by pro-inflammatory cytokines such as interleukin-1 β , tumor necrosis factor- α , and lymphotoxin, and also by bacterial endotoxin, and interferon- γ . Selectins are important for the initial tethering of neutrophils along the endothelial cell surface³⁷. If other adhesion molecules on the endothelium and leukocytes are also expressed or activated, then firm adhesion and transmigration will occur and the inflammatory response will be in full force. Since E-selectin is not constitutively expressed or stored, its expression on endothelial cells indicates an active induction. E-selectin expression on activated endothelial cells occurs within 1-4 hours after activation by pro-adhesive cytokines, with expression lasting approximately 24 hours^{7,8,37}. In contrast P-selectin is translocated from endothelial cell Weibel-Palade bodies²⁶ within minutes following stimulation with thrombin or histamine¹⁹. Although not the earliest indicator of endothelial activation, E-selectin may be the most specific marker of endothelial injury as it is expressed only on activated endothelium. Clinical studies have shown elevated levels of the soluble form of E-selectin (sE-selectin) in patients with septic shock prior to development of acute lung injury and the associated permeability capillary leak^{9,29,34}.

Table 1.
Biological properties of selectin adhesion molecules.

Property	E-selectin	P-selectin	L-selectin
Distribution	Endothelial cells	Endothelial cells Platelets	PMNs Monocytes Lymphocytes
Activators	Interleukin-1 TNF- α Lymphotoxin Interferon- γ Endotoxin Hypoxia? Mechanical stress	Thrombine Histamine H_2O_2 Hypoxia?	constitutively expressed
Target cells	PMNs Monocytes Lymphocytes	PMNs Monocytes Lymphocytes	Endothelial cells
Duration of Expression	1 to 24 hours	1 to 15 minutes	NA

Eldridge *et al.*¹² measured sE-selectin, interleukin-1 β (IL-1 β), TNF- α , and von Willebrand factor antigen (vWF-ag) in mixed venous and arterial blood from HAPE-S and control subjects following normoxic and hypoxic exercise at sea level and after two days at 3810m altitude. Figure 1 shows sE-selectin values for both groups before and after normoxic ($P_1O_2=147$ Torr) and hypoxic ($P_1O_2=89$ Torr) exercise at sea level

and 3810m altitude. Soluble E-selectin values for the control subjects did not change with either exercise bout at sea level. However, HAPE-S subjects had an increase in circulating sE-selectin levels following the hypoxic exercise, though this change did not reach statistical significance ($p=0.063$). Cytokines and vWF-ag showed no consistent pattern in either group. In addition to the sE-selectin findings, the HAPE-S subjects showed significantly greater pulmonary vascular pressures ($p<0.001$)¹⁴, and demonstrated greater ventilation perfusion mismatch ($p<0.05$)³¹ than did the controls with each P_1O_2 .

After two days at 3810m altitude (University of California, Barcroft Research Station White Mountain, CA) the subjects were studied again. At altitude the subjects were given 33% oxygen during the normoxic exercise providing an inhaled $P_1O_2=144$ Torr whereas ambient air provided an inhaled $P_1O_2=91$ Torr. Soluble E-selectin (Fig. 1) values were significantly elevated ($p<0.05$) in the HAPE-S subjects following both normoxic and hypoxic exercise at altitude. These subjects also had marked elevations in pulmonary vascular pressure ($p<0.001$)¹⁴ and demonstrated a significantly greater gas exchange dysfunction ($p<0.05$)³¹ with both exercise bouts as compared to controls. Interestingly, control subjects had significantly elevated levels of sE-selectin following hypoxic exercise. These findings were associated with a significant increase ($p<0.05$ as compared to sea level) in wedge pressure and pulmonary vascular resistance¹⁴ as well as a greater gas exchange abnormality³¹. No changes were seen with normoxic exercise. Again the cytokine and vWF-ag values showed no particular pattern in either group.

Adhesion Molecules and Cytokines with Exposure to Altitude

Surprisingly, simple exposure to altitude appears to stimulate induction and expression of E-selectin^{11,12,15,24}. Figure 2 shows resting individual and mean sE-selectin

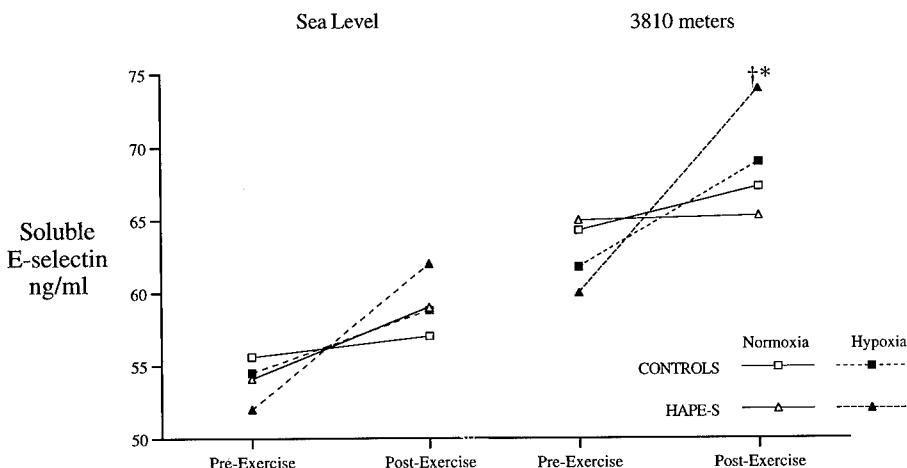


Figure 1 Soluble E-selectin levels before and after exercise in 9 healthy controls and 7 individuals with a history of high altitude edema. Exercise duration was approximately 40 minutes, with peak effort 85% maximal for the given P_1O_2 . Normoxia had $P_1O_2=147$ Torr; hypoxia had $P_1O_2=91$ Torr. Hypoxic exercise at 3810m resulted in a significant increase in circulating soluble E-selectin. $\dagger p=0.05$ different than pre-exercise. $*p=0.05$ different than control subject values.

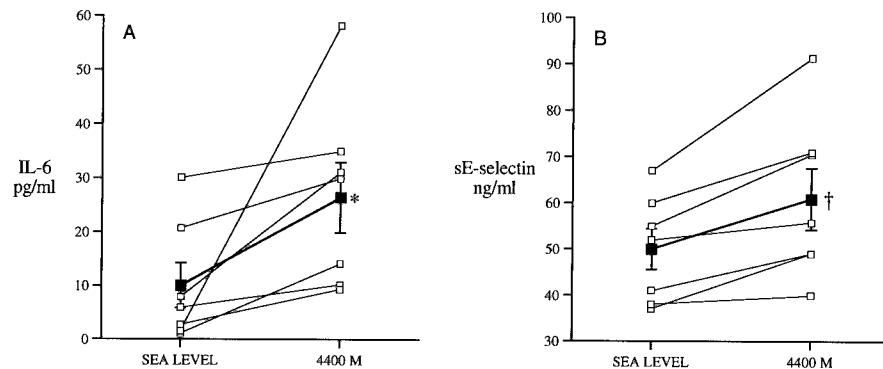


Figure 2 Interleukin-6 (IL-6) (A), and soluble E-selectin (B) levels in 7 climbers on Denali. Altitude values were venous samples taken 12 hours after arrival at 4400m following a 4 day ascent. E-selectin levels increased with ascent ($†p=0.007$). IL-6 levels were higher at altitude but did not reach statistical significance ($*p=0.06$). No subject developed significant altitude illness as assessed by physical examination and AMS scoring.

data for controls and HAPE-S subjects at sea level and 3810m. sE-selectin values increased significantly in both control ($p=0.007$) and HAPE-S subjects ($p=0.001$) with exposure to altitude. No changes were noted in the cytokines or vWF-ag. Dechaux *et al.*¹² measured sE-selectin in five healthy volunteers at sea level and after five days at 6542m altitude, and showed a significant ($p<0.001$) rise in circulating sE-selectin. Grissom *et al.*¹⁵ followed sE-selectin, TNF- α , vWF-ag, and P-selectin levels in seven climbers on Denali and demonstrated a significant elevation in sE-selectin upon arrival at the 4400m camp as compared to sea level (ascent time of 4 days). No changes were seen in P-selectin, vWF-ag and TNF- α .

Interleukin-6 (IL-6) is another pro-inflammatory cytokine that is elaborated from a large number of cell types (including alveolar macrophages and endothelial cells) in response to tissue injury, inflammation and infection. Although it is an autocrine agent, IL-6 appears to have a greater systemic presence, and longer serum half-life than IL-1 β and TNF- α . Interleukin-1 β and TNF- α will stimulate release of IL-6 from endothelial cells and macrophages. Recently, IL-6, sE-selectin and L-selectin levels were measured in climbers on Denali^{13,25}. In seven climbers studied at sea level and 4400m (4 day ascent) both IL-6 ($p=0.06$) and sE-selectin ($p=0.007$) increased at altitude (Fig. 3). Seven additional climbers were studied upon arrival at 4400m and then again after nine days at that altitude and showed no changes in either IL-6 or sE-selectin values over the study period. These data suggest that endothelial cells have been activated and are expressing E-selectin on their cell surfaces.

L-selectin is expressed on cytokine-activated leukocytes^{7,37}. Soon after expression on the cell surface L-selectin is proteolytically cleaved and its soluble form (sL-selectin) is released into the circulation³⁷. It is not completely clear why sL-selectin is released from the activated neutrophil, but it may play a role in modulating the inflammatory response by binding to sialylated Lewis-X (SLe x) ligand on the neutrophil³⁷. SLe x is constitutively expressed on leukocytes and is the binding ligand for the selectin adhesion molecules during the tethering phase of leukocyte-endothelial cell adhesion and transmigration. If sL-selectin levels are elevated then leukocytes have also been activated. In the Denali study L-selectin levels were unchanged with ascent

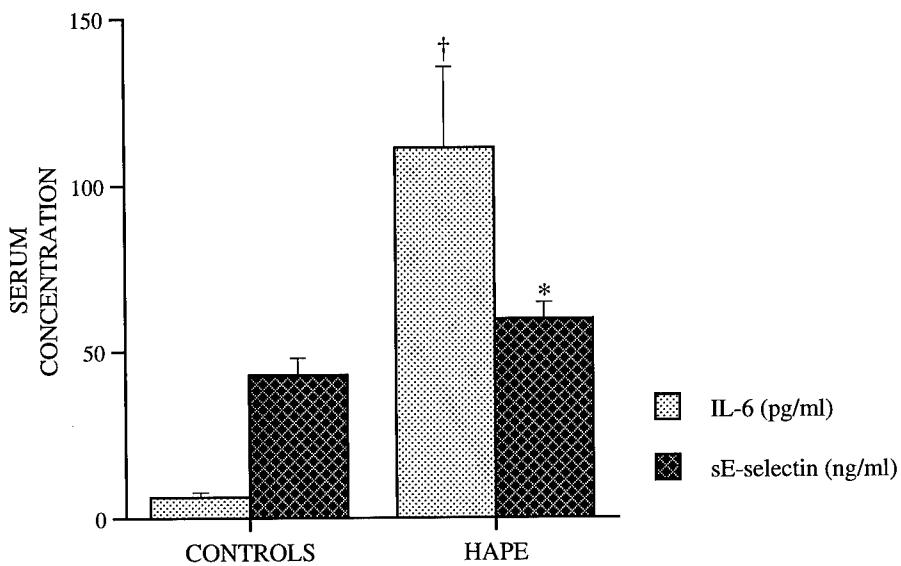


Figure 3 Serum concentrations of IL-6 and E-selectin levels in 7 healthy climbers at 4400m (Denali) and 4 climbers with high altitude pulmonary edema. IL-6 levels were markedly elevated in the HAPE victims ($\dagger p=0.0001$), whereas E-selectin levels were higher but the difference did not reach statistical significance ($*p=0.062$).

to altitude or after nine days at altitude. The lack of significant sL-selectin elevation implies a limited activation of leukocytes and a modulation of the inflammatory response.

Cytokines and Adhesion Molecules in HAPE

Very little data is available on cytokines and adhesion molecule activation in HAPE. Grissom *et al.*¹⁵ measured vWF-ag, P-selectin and sE-selectin in five climbers with acute mountain sickness and four others with acute HAPE. P-selectin and sE-selectin values were not different than those found in non-ill climbers at the same altitude. They did report elevated levels of vWF-ag similar to those reported previously^{4,5}. Eldridge *et al.*¹³ also measured cytokines and adhesion molecules in HAPE victims on Denali. Table 2 shows the results of that study. They found elevated levels of IL-6 ($p<0.002$), and sE-selectin ($p=0.062$), sL-selectin, TNF- α , or intercellular adhesion molecule 1 (ICAM-1) as compared to well climbers.

Discussion

An inflammatory-mediated mechanism is clearly involved in the pathogenesis of high altitude pulmonary edema as demonstrated by postmortem findings, lung lavage analysis and elevated levels of vWF-ag, sE-selectin, and IL-6. The temporal relationship between inflammatory activation and pulmonary endothelial injury with HAPE remains a mystery. We have demonstrated that sE-selectin levels are elevated with exposure to altitude indicating an early activation of endothelial cells. Of great interest is the mechanism of endothelial cell activation with altitude exposure. *In vitro* studies have shown expression of platelet activating factor (PAF)², E-selectin³⁵, and P-selectin³⁰ on human umbilical vein endothelial cells (HUVEC) upon reoxygenation

following anoxic stress. When the cells were examined during anoxia, minimal activation was noted. Free radical scavengers appear to limit the adherence of leukocytes following reoxygenation suggesting that oxygen-derived free radicals may be the activators for the adhesion molecule expression³⁰.

Table 2.
Cytokines and adhesion molecules in HAPE.

	Controls n=7	HAPE n=4	p value
IL-6 (pg/ml)	6.2±3.9	111.3±48.0	0.0001
TNF- α (ng/ml)	71.5±78.0	53.5±29.3	0.676
E-selectin (ng/ml)	42.7±13.8	59.6±10.2	0.062
L-selectin (ng/ml)	1515±355	1269±248	0.313
ICAM-1 (ng/ml)	259.0±42.0	269.0±10.8	0.707

IL-6, interleukin-6, TNF- α , tumor necrosis factor-a, ICAM-1, intracellular adhesion molecule-1. p values represent differences from control subjects with sera obtained after 9 days at 4400m.

If the elevated E-selectin values seen with ascent to altitude are due to hypoxic reoxygenation injury then the most likely sources are the post-capillary venule or pulmonary arterial endothelial cells. During high level exercise (85% maximal effort) at altitude ($P_1O_2=91$ Torr) mixed venous oxygen tensions can be extremely low ($PvO_2=16$ Torr)¹⁴, and if this hypoxic stress is prolonged, hypoxic reoxygenation injury could occur with liberation of oxygen-derived free radicals. This may explain the elevated E-selectin levels seen following hypoxic exercise in HAPE-S individuals, but the origin of the E-selectin with simple ascent to altitude remains unclear.

Studies during acute HAPE did not demonstrate significant endothelial activation or injury shortly after treatment with oxygen or descent. Although soluble E-selectin levels were not significantly different ($p=0.062$)^{13,25} than in well climbers at the same altitude, the sample size was very small and further studies are needed to determine the relationship. Even though recovery from HAPE is typically rapid with appropriate treatment it is surprising that greater evidence of endothelial injury was not found. Since cytokine and adhesion molecule levels were not obtained prior to the development of HAPE it is impossible to determine the changes in each individual. P-selectin values were also unchanged, but the expression of P-selectin is an extremely early event and would likely be missed during the treatment period³⁷. The only evidence of an ongoing inflammatory process is the elevated level of the pro-inflammatory cytokine IL-6. Interleukin-6 is probably secreted by the activated macrophages found in the alveolar space in HAPE, although its role at this stage is unclear.

We do not know how the endothelial cells are activated with hypoxic stress or during acute HAPE. Moreover, we do not even know which vascular bed is being activated and releasing the soluble selectins. Serial measurements of E-selectin, P-selectin, and L-selectin may provide insight into the nature of the rapid resolution and limited residual lung injury seen with HAPE. Furthermore, analysis of receptor acti-

vation on leukocytes found in the alveolar fluid may tell us how these inflammatory cells are recruited in such abundance to the alveolar space. Carefully designed studies during hypoxic exercise in HAPE-S subjects need to be performed to determine the timing of cytokine stimulation and endothelial activation. It would also be interesting to attempt blockade of endothelial activation with steroids or non-steroidal anti-inflammatory agents. Clearly, further studies addressing these and other questions need to be conducted before we can fully understand the pathogenesis of high altitude pulmonary edema.

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CHAPTER 21

ELEVATED WEDGE PRESSURE IN HAPE- SUSCEPTIBLE SUBJECTS DURING EXERCISE

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Introduction

High altitude pulmonary edema (HAPE) is an uncommon, potentially fatal syndrome seen at altitudes above about 8,000 ft. and characterized by arterial O₂ desaturation, cough and shortness of breath excessive for the altitude with pulmonary rales on physical exam²². There are chest x-ray findings of patchy infiltrates due to alveolar filling, and after diagnosis is made, no evidence of structural or functional left ventricular abnormalities is found. Risk factors are excessive rate of ascent and exercise, particularly in combination. Respiratory infection is not implicated, but can be a cause of mistaken diagnosis.

The most widely regarded hypothesis underlying HAPE has been related to increased pulmonary microcirculatory pressure caused by hypoxic vasoconstriction. Via the balance of forces controlling transcapillary fluid movement, this raises fluid efflux into the pulmonary interstitium and eventually leads to alveolar edema. This mechanism requires the uneven development of hypoxic vasoconstriction, because if vasoconstriction were uniform, the microcirculation would be protected from high pressures by the very upstream constriction that causes hypoxic pulmonary hypertension in the first place¹⁰.

Recent advances in our understanding of the pathogenesis of HAPE include the remarkable bronchoalveolar lavage findings of Schoene¹⁸ that demonstrated a high protein, cellular alveolar fluid in acute HAPE implying much more than a simple increase in transcapillary fluid filtration. Thus, there appears to be a strong inflammatory component. In addition, West and Mathieu-Costello²⁴ believe that high microvascular pressures directly disrupt the blood gas barrier, allowing escape of cells and fluid into the alveoli, and thus contributing to HAPE.

A strongly preserved theme over the years has been the claim that left ventricular filling pressures (as estimated from pulmonary artery wedge pressure measurements)

are not elevated in HAPE^{9,10,22}. This has been troubling for two reasons. First, such data come from patients who already have clinically obvious HAPE and are therefore lying supine and at rest. Given the conditions under which HAPE usually develops, namely, heavy exercise, it would seem necessary to measure the wedge pressure during exercise before dismissing the wedge pressure as not elevated or non-contributory to HAPE. Second, a normal wedge pressure demands a large pressure drop from the microvasculature to the pulmonary veins (if HAPE is to be the result of high capillary pressure in the first place) and whether this is easily explained is debatable. On the other hand, if wedge pressure were elevated (while HAPE was developing during exertion), it becomes much easier to imagine how capillary pressure is elevated. Moreover, it would remove the need for postulating non-uniform hypoxic vasoconstriction in the normal lung, itself a somewhat troubling concept.

It therefore seems important to know whether wedge pressures *during exercise* are unduly elevated in HAPE-susceptible individuals, a question not previously addressed.

METHODS

Subjects:

Sixteen currently healthy, moderately fit subjects were studied. All had been to altitudes of 4,000m or more on several occasions. Nine had no clinical evidence of any respiratory disease while at altitude (CONTROLS) while seven (HAPE-S) had previously suffered clearly physician-documented HAPE with the above-mentioned clinical picture that resolved within hours of descent and/or supplemental O₂. Their anthropometric data are shown in Table I and demonstrate good matching for the usual physiological features, with one exception: the CONTROLS had a mean forced vital capacity 14% greater than predicted while HAPE-S had normal volumes.

Protocol:

Each subject underwent four incremental exercise tests, each test beginning with resting measurements followed by successive steps to about 25, 50, and 90% of peak workload (determined previously for each condition of exercise). Two of the four tests took place in San Diego at sea level, and two at 3810m at the Barcroft Laboratory of the University of California White Mountain Research Station after 36-48 hours of residence at that altitude. At each location, one test used room air (FIO₂=148 torr at San Diego, 91 torr at White Mt.) and the other an altered FIO₂ (12% at sea level, 33% at White Mt., yielding PIO₂ values of about 91 and 148 torr respectively). The two tests at each location were separated by at least 30 min and the order of PIO₂ randomized amongst subjects. The White Mt. component was performed about two weeks after the San Diego study. In each test, at rest and each exercise level, data were obtained in duplicate, and consisted of:

- A. Measures of pulmonary gas exchange: arterial, mixed venous and mixed expired O₂, CO₂ and inert gas levels (for the multiple inert gas elimination technique) using standardized, published methodology^{2,4}. Pulmonary arterial temperature was also recorded.
- B. Measurement of ventilation by Tissot-calibrated Wright's respirometer.
- C. Measures of pulmonary arterial pressure (PAP) and pulmonary artery occlusion (or wedge) pressure (PAOP).

Table I: Anthropometric Data

HAPE-Susceptible Subjects					
	Age yr	Height cm	Weight kg	FVC	FEV ₁ /FVC
				% pred.	% pred.
MEAN	34.4	177.1	76.0	102	104
SE	2.6	2.1	4.0	4	2

CONTROL Subjects					
	Age yr	Height cm	Weight kg	FVC	FEV ₁ /FVC
				% pred.	% pred.
MEAN	29.4	177.2	76.0	114	99
SE	1.7	2.9	3.3	5	4

Only FVC is different between groups, $p = 0.05$

Calculations:

The inert gas data were transformed into the distribution of ventilation/perfusion ratios² by standard algorithms. Cardiac output was calculated from measured O_2 uptake and the arterial and pulmonary arterial O_2 concentrations by the Fick principle. An estimate of pulmonary capillary pressure was computed as the simple average of PAP and PAOP, as suggested by the results of Nagasaka *et al.*,¹³. In some studies, it is suggested that capillary pressure may be closer to PAP than PAOP²⁵ but since this would be difficult or impossible to establish in man during exercise and our primary interest is in comparing HAPE-S and CONTROL groups, the choice of a simple average appears reasonable. O_2 uptake and CO_2 output were computed using standard equations and expired gas O_2 and CO_2 levels and minute ventilation.

Statistical analysis was based on repeated measures analysis of variance and post-hoc t-tests using the Bonferroni correction for multiple comparisons. Analysis of co-

variance was used to separate the influence of intersubject variability in spirometric indices on gas exchange parameters.

RESULTS:

The two groups differed in several respects at San Diego; these differences disappeared at White Mt. Hence, only the data from San Diego are presented in detail.

San Diego Data

Table I shows mean spirometric differences between the two groups. Points of importance are a) mean FVC values in CONTROLS were greater than predicted by 14% ($p=0.05$), b) mean values of FEV_1/FVC were not different between groups and c) there was considerable intersubject variance in this ratio in both groups.

Table II shows gas exchange variables at each exercise level. Note that work rates and $\dot{V}O_2$ at each rest and exercise level were not different between the groups, suggesting similar fitness and exercise responses in general. Both cardiac output and minute ventilation were slightly but significantly ($p<0.05$) lower in HAPE-S than CONTROLS at the highest work rate in both normoxia and hypoxia.

Pulmonary vascular pressures were higher in HAPE-S than CONTROLS especially at moderate and heavy exercise ($p<0.05$). This was true for both PAP and PAOP and at both FIO_2 levels. Pulmonary vascular resistance ((PAP-PAOP)/QT) was higher in HAPE-S at each workload, and mean capillary pressure ((PAP+PAOP)/2) was also higher than in CONTROLS ($p<0.05$ each). These relationships are shown in Figure 1.

Indices of $\dot{V}A/\dot{Q}$ mismatching showed concordance with many previous studies^{4,7,8,20}. Thus, $\dot{V}A/\dot{Q}$ inequality increased significantly with exercise and this was seen for both subject groups in both FIO_2 environments. Of particular note was the greater degree of exercise-induced $\dot{V}A/\dot{Q}$ inequality in HAPE-S than CONTROL subjects at each FIO_2 . This difference was significant ($p=0.05$) after allowing for variance contributed by intersubject variability in FEV_1/FVC in each subject group by means of analysis of covariance.

White Mountain Data

Spirometry was not repeated at White Mt. As at sea level, work rates and $\dot{V}O_2$ were not different between the two subject groups at either PIO_2 at any workrate. The CONTROL subjects were no longer different from HAPE-S with respect to any of the principle variables—those related to pulmonary vascular pressures, cardiac output, ventilation, or gas exchange.

The only significant differences between the data at San Diego and those at White Mt. were in the estimate of O_2 pulmonary diffusing capacity (DLO_2), which was some 30% lower in both subject groups at White Mt.

Exercise still produced an increase in $\dot{V}A/\dot{Q}$ mismatch, but of similar degree in both groups.

DISCUSSION

The principal observations in this study, divided into those seen at San Diego and those seen at White Mt. are summarized briefly below.

TABLE II. Summary of cardiopulmonary variables during rest and during exercise at sea level

	NORMOXIA (PB = 755 Torr, PIO2 = 148 Torr)		HYPOXIA (PB = 755 Torr, PIO2 = 89 Torr)	
	Rest	Exercise (% Watt max)	Rest	Exercise (% Watt max)
	0	85%	0	85%
Workrate, Watts				
HAPE (n=7)	0	282.6 ± 16.9	0	224.6 ± 15.2†
CON (n=9)	0	283.0 ± 46.0	0	217.3 ± 40.4†
VO2, ml/min	383 ± 77 394 ± 73	3534 ± 531 3763 ± 637	376 ± 85 449 ± 157	2707 ± 335† 2932 ± 311†
VE, l/min (BTPS)	11.6 ± 2.8 13.0 ± 3.1	120.4 ± 14.9 136.6 ± 19.0	14.6 ± 4.4 22.7 ± 19.7	115.2 ± 23.4 121.8 ± 23.8
QT, l/min	6.4 ± 0.9 6.7 ± 1.1	22.3 ± 2.5* 23.9 ± 3.6	6.8 ± 1.4 8.4 ± 2.5	22.0 ± 2.3* 24.0 ± 3.5
HR, beats/min	77.1 ± 18.5 79.2 ± 14.3	176.8 ± 10.6 183.7 ± 13.0	90.4 ± 19.1 93.3 ± 14.4	174.4 ± 10.3 177.1 ± 12.4†
PaO2, Torr	108.6 ± 5.7 109.4 ± 11.2	107.4 ± 4.8 104.4 ± 9.9	52.5 ± 6.2† 54.3 ± 10.0†	43.2 ± 2.4† 44.3 ± 3.6†
PaCO2, Torr	32.9 ± 3.1 33.7 ± 3.9	34.3 ± 2.3 33.7 ± 3.0	29.6 ± 3.7 29.5 ± 6.9	28.5 ± 1.4† 29.2 ± 3.5†
A-a PO2, Torr	-2.5 ± 3.2 -1.4 ± 6.2	7.6 ± 3.6 10.9 ± 7.3	-0.6 ± 2.7 1.2 ± 4.0	13.0 ± 2.9† 12.9 ± 2.7
PvO2, Torr	36.5 ± 3.2 36.6 ± 1.2	22.9 ± 3.4 23.7 ± 3.1	30.7 ± 2.2† 29.1 ± 6.0†	14.9 ± 2.9† 17.0 ± 3.6†
Lactate, mmol/l	2.7 ± 2.5 1.6 ± 1.6	8.7 ± 0.8 8.7 ± 2.4	2.0 ± 1.4 1.7 ± 1.2	10.1 ± 2.7 9.6 ± 3.3
log SDQ	0.41 ± 0.08 0.45 ± 0.07	0.62 ± 0.21* 0.54 ± 0.14	0.41 ± 0.10 0.48 ± 0.14	0.71 ± 0.40* 0.62 ± 0.28
log SDV	0.41 ± 0.08 0.45 ± 0.07	0.47 ± 0.06 0.47 ± 0.04	0.41 ± 0.08 0.46 ± 0.09	0.45 ± 0.08 0.48 ± 0.06
%Q, in VA/Q<0.1	0.2 ± 0.2 0.4 ± 0.7	1.0 ± 1.0 0.6 ± 0.7	0.3 ± 0.2 0.6 ± 0.8	1.6 ± 2.1 1.0 ± 1.2
A-aPO2 pred, Torr	9.1 ± 3.6 9.7 ± 4.5	14.4 ± 7.0 11.6 ± 4.5	3.5 ± 2.2† 4.0 ± 1.3	3.4 ± 2.1† 4.0 ± 1.3†
DLO2, ml(min.Torr)-1	-- --	-- --	-- --	107.6 ± 15.0 122.2 ± 37.4

Values are means ± SD. PB, barometric pressure; PIO2, inspired PO2; VO2, oxygen uptake; VE, ventilation; QT, cardiac output; HR, heart rate; PaO2, arterial PO2; PaCO2, arterial PCO2; A-aPO2, alveolar-arterial PO2 difference; PvO2, mixed venous PO2; Lactate, blood lactate concentration; log SDQ and log SDV, log standard deviation of perfusion and ventilation distributions, respectively; %Q in VA/Q<0.1, percent of perfusion to lung units with VA/Q<0.1; A-aPO2 pred., alveolar-arterial PO2 predicted from recovered VA/Q distributions; DLO2, O2 diffusing capacity of lung. * = significantly different from CON (p < 0.05); † = significantly different from normoxia (p < 0.05).

San Diego Data

1. Lung volumes of control subjects were significantly (14%) greater than predicted; those of the HAPE-S group were not (102% of predicted).
2. PAP, PAOP, pulmonary vascular resistance and estimated pulmonary capillary pressure were all higher in HAPE-S, especially at heavy exercise.
3. Peak exercise ventilation and cardiac output were lower in HAPE-S subjects.
4. Exercise-induced $\dot{V}A/\dot{Q}$ mismatch was seen in both groups, was greater in hypoxia, and was significantly exaggerated in HAPE-S subjects.

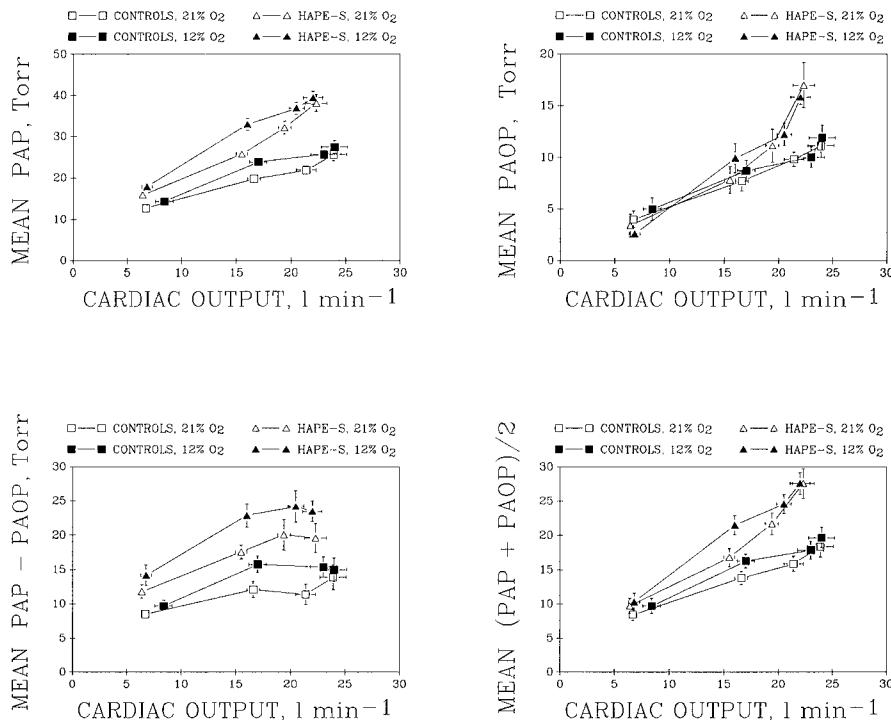


Figure 1 Relationship between pulmonary vascular pressures and flow in CONTROL and HAPE-susceptible subjects at sea level. PAP is pulmonary artery pressure, PAOP is pulmonary artery occlusion (or wedge) pressure. The difference (lower left panel) indicates pulmonary driving pressure, and the mean (bottom right panel) is one estimate of pulmonary capillary pressure. In all panels, data for HAPE-susceptible subjects exceeds those of CONTROL subjects at moderate and high intensity exercise, with additional effects of hypoxia as shown.

White Mountain Data

1. Differences between the subject groups disappeared. This was true for hemodynamic and gas exchange variables.
2. Pulmonary O₂ diffusing capacity was reduced from San Diego levels in both groups of subjects, by about 30%.

This study addresses a number of interconnected but conceptually separate issues that will be discussed in turn:

- A. Role of PAOP in HAPE, and mechanism of its elevation.
- B. Exaggerated $\dot{V}A/\dot{Q}$ inequality in HAPE-S subjects.
- C. Mechanism of $\dot{V}A/\dot{Q}$ inequality during exercise.
- D. Potential role of lung size in pathogenesis of HAPE.
- E. Incidence of HAPE.
- F. Effects of 36-48 hours of altitude residence on exercise responses.

A. Role of PAOP in HAPE

Exaggerated hypoxic pulmonary vasoconstriction is well-established in HAPE-S subjects^{3,11,12} and the present studies are concordant with these observations, extend-

INCREASE IN PRESSURES FROM REST TO EXERCISE

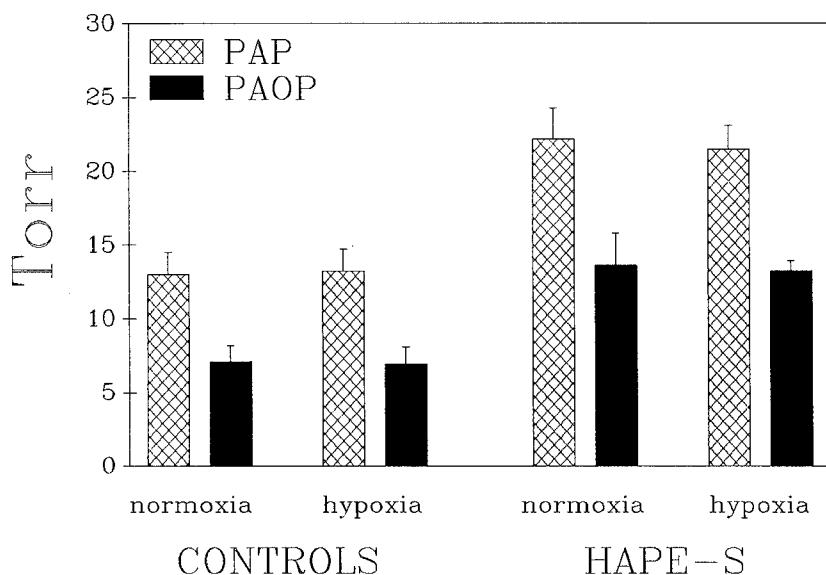


Figure 2 Contribution of pulmonary artery occlusion pressure to pulmonary artery pressure in CONTROL and HAPE-susceptible subjects at sea level. The graph shows increases in indicated pressures from rest to exercise and demonstrates that PAOP accounts for more than half of the rise in pulmonary arterial pressure in both groups.

ing them to a higher exercise level than have previous authors. However, the key new information is that while resting PAOP is not greater than in control subjects, it is clearly greater at higher exercise levels at sea level (Fig. 1). Figure 2 illustrates the degree to which the elevated PAOP is responsible for the elevated PAP and shows that more than half of the exercise-related increase in PAP is attributable to the rise in PAOP. Consequently, this is new evidence of the likely elevation in pulmonary microvascular pressure, and this in turn complements current thoughts on the role of such pressure changes in the genesis of HAPE.

The question of why PAOP is elevated needs to be addressed. Several possibilities exist, although none can be distinguished by the present data. First, pulmonary *venous* hypoxic vasoconstriction could be exaggerated in HAPE-S subjects, just as conventional pulmonary *arterial* hypoxic pressor responses are greater in this group. Second, HAPE-S subjects could be at the end of the normal distribution of left ventricular compliance such that higher ventricular filling pressures are required. Third, the high right ventricular pressures in HAPE-S subjects could directly impede left ventricular function, especially with the intact pericardium limiting ventricular distension. This mechanism is in general well established^{6,19} although its role in HAPE is speculative. However, it suggests a circular feedback problem: a higher right ventricular pressure impedes the left ventricle, which requires a higher filling pressure and raises PAOP. This in turn requires a higher PAP, right ventricular pressure and

thus further impedes the left ventricle. This could be tested in an animal model of HAPE by pericardectomy.

A fourth possibility is especially intriguing. Note from Table I that lung volume was less in HAPE-S subjects. Thus one could postulate a reduced pulmonary vascular cross-sectional area and as a result, higher vascular pressures throughout the pulmonary tree.

Whichever the mechanism, to the extent that increased microvascular pressures are required to induce HAPE, the finding of an elevated PAOP during exercise is very likely a significant contributing factor to the disease. It provides an alternate hypothesis to that of non-uniform hypoxic vasoconstriction as a mechanism for explaining elevated microvascular pressures and remains compatible with the well-known finding of normal PAOP in resting supine patients with HAPE.

B.&C. Exaggerated $\dot{V}A/\dot{Q}$ Inequality in HAPE-S Subjects and Mechanism of $\dot{V}A/\dot{Q}$ Mismatch During Exercise

We have long postulated that exercise-induced $\dot{V}A/\dot{Q}$ mismatch may be due to mild, presumably interstitial, pulmonary edema^{16,17,20}. While unequivocal evidence remains impossible to obtain due to technical limits of approaches that could directly measure interstitial edema, the relationship to PAP²⁰, the exaggerated $\dot{V}A/\dot{Q}$ inequality in acute hypoxia⁴, the lack of spirometric abnormalities during exercise²³ and the relatively long time course of $V/A/Q$ abnormalities after exercise¹⁷ all point to edema as a likely cause. It is also known that marathon running produces reductions in lung volume and diffusing capacity^{1,14}, and in the pig, perivascular edema develops during heavy exercise¹⁶.

It is therefore a small step to postulate that HAPE-S subjects, with their higher PAP responses to hypoxia and exercise, might develop more exercise-induced $\dot{V}A/\dot{Q}$ mismatch than controls. In fact, this hypothesis was originally the driving motivation for the study. The data support this hypothesis as described in the results.

Unlike prior subject groups studied to evaluate $\dot{V}A/\dot{Q}$ relationships during exercise, the present subjects were difficult to identify due to the requirements of having been to high altitudes and either developed or not developed HAPE. Consequently, we had to accept some variance in spirometry (Table I). This however was done only after excluding any respiratory history of significance (other than HAPE). Independent regression of $V/A/Q$ inequality parameters against FEV_1/FVC showed a weak but appropriate relationship—inequality increasing as FEV_1/FVC decreased. As mentioned, there were no systematic differences in FEV_1/FVC between subject groups, so that the FEV_1/FVC influence cannot be argued as anything related to HAPE. It is therefore thought reasonable to take account of this covariant when determining significance of differences in $\dot{V}A/\dot{Q}$ mismatch between the two subject groups.

The somewhat lower cardiac output and minute ventilation of HAPE-S subjects yet higher pulmonary vascular pressures is evidence that the mere increase in gas or blood flowrates in the lung is not *per se* the cause of exercise-induced $V/A/Q$ inequality. Figure 3 illustrates that in addition, $\dot{V}A/\dot{Q}$ mismatch is closely related to the PAP reached during exercise: HAPE-S subjects do not show greater $\dot{V}A/\dot{Q}$ mismatch at the same PAP than controls, but develop more $\dot{V}A/\dot{Q}$ inequality associated with a higher PAP. These results contrast with the separation of $\dot{V}A/\dot{Q}$ inequality from cardiac output and minute ventilation also shown in figure 3. They remain conceptually consistent with previous data that show persistent $\dot{V}A/\dot{Q}$ mismatch after exercise, even after cardiac output and minute ventilation have returned to near baseline levels¹⁷.

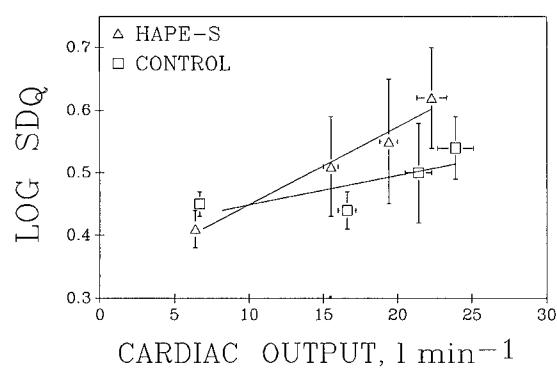
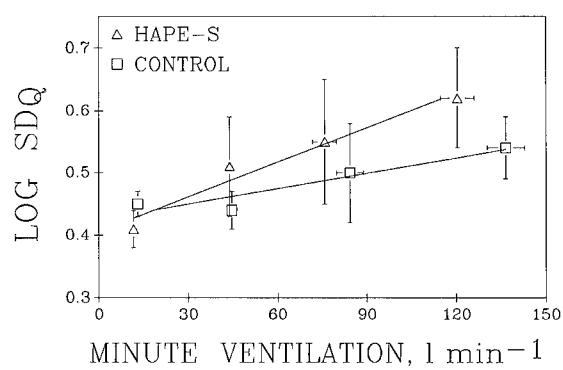
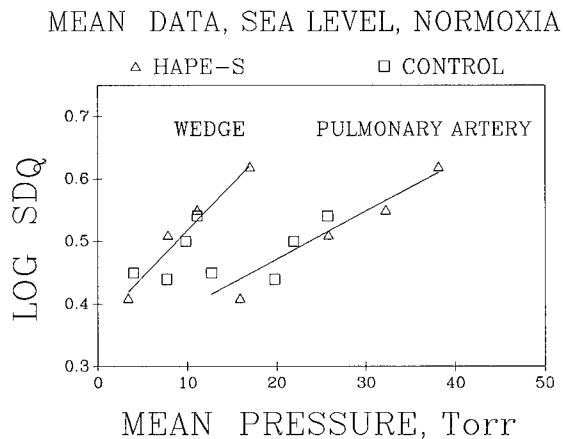


Figure 3 Relationship between ventilation/perfusion inequality (measured by second moment of the perfusion distribution, log SDQ) and pulmonary vascular pressures, minute ventilation, and cardiac output. Each data point represents the average of all subjects at a given exercise level, in normoxia at sea level. Ventilation/perfusion inequality relates closely to vascular pressures, but not to minute ventilation or cardiac output. Error bars excluded from top panel for clarity.

D. Lung Size and HAPE

The preceding has emphasized differences between HAPE-S and control subjects but has not dwelt on the interesting observation that by and large, the physiological responses of our HAPE-S subjects are quite similar to those of *non-selected* groups of subjects studied using similar methods and previous protocols. Thus, figure 4 compares the PAP and PAOP responses (in normoxia at sea level) of our present group with those of two prior studies^{5,20}. It is evident that *maximal* PAP and PAOP are very similar amongst the HAPE-S subjects of the present study and the unselected subjects of previous studies. In contrast, maximal PAP and PAOP of the control group is unusually low.

Recall that lung volumes of HAPE-S subjects were less than those of control subjects (Table I) but that in absolute terms, HAPE-S had normal volumes while control subjects had *larger* than predicted lung volumes, by 14%.

What emerges from this series of observations is that compared to *unselected* normal subjects, it is the control subjects, not the HAPE-S group, that appear to differ in physiological exercise response and lung size. We therefore postulate that there may be little about HAPE-S subjects that renders them susceptible to HAPE. Rather, we may have identified factors that *protect* subjects from HAPE: a large lung that may afford an intrinsically lower pulmonary vascular resistance due to its structure. Lower vascular pressures in turn damage the microvasculature less and reduce the incidence of HAPE.

Anecdotal personal communications from Dr. Peter Hackett are well in line with this concept, based on clinical experiences with a large number of climbers in the field. Further, the poster by the group of Dr. Peter Bärtsch at this year's Hypoxia meeting, which also examined HAPE-S and resistant subjects, also suggests a 10% larger lung volume in the resistant subjects¹⁵.

This hypothesis deserves to be further tested in a much larger study of HAPE development in the field.

E. The Incidence of HAPE

The foregoing hypothesis indicates that HAPE-S subjects in general have physiological responses that are not excessive compared to unselected normal volunteers. This has a potentially disturbing implication for the incidence of HAPE. It would suggest that in essence all normal subjects are liable to develop HAPE if the physiological stresses of combined hypoxia and exercise are severe enough.

The relatively low incidence of HAPE at common North American altitudes seems at odds with this view of HAPE pathogenesis. However, it is virtually certain that prior to clinically obvious HAPE, there must be a subclinical level that is more frequent than the full-blown clinical picture. If not looked for, it cannot be documented. Moreover, conventional studies such as arterial blood gases and chest x-ray may not reveal early manifestations and more subtle methodologies might be required. More compelling however, is the outcome of studies carried out during Operation Everest II in 1985. At 20,000 ft. (PB=347 torr), subjects were being decompressed perhaps too rapidly, and most developed several features of cerebral or pulmonary involvement in mountain sickness. When studied during exercise, six of seven showed dramatic degrees of $\dot{V}A/Q$ inequality using the multiple inert gas elimination technique²¹. These raised common indices of inequality to levels normally seen only in intensive care settings in patients with moderate to severe lung disease, and frank shunting was

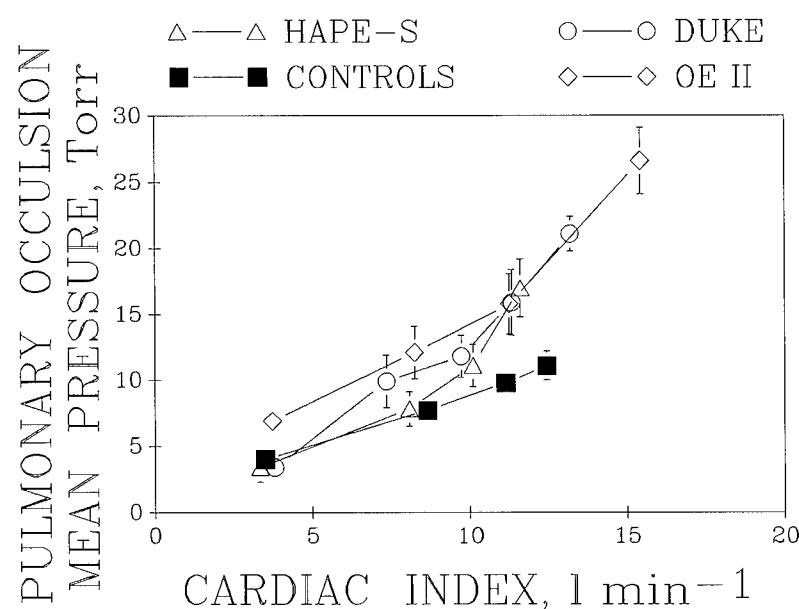
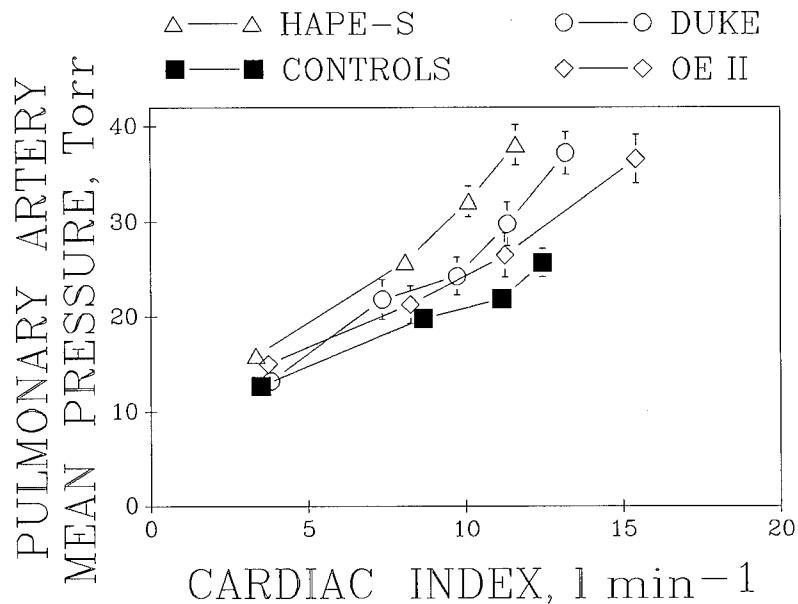


Figure 4 Pulmonary vascular pressures (sea level, normoxia) compared to values from the literature. "Duke" refers to reference (20) and "OEII" refers to reference (21). Notice that maximum pulmonary artery pressure reached similar levels in HAPE-susceptible subjects as in the two prior studies, but that pulmonary artery pressure in the present control group was much lower at peak exercise. The same is evident for pulmonary artery occlusion pressure as shown in the lower panel.

observed, which together with pulmonary rales, confirmed the impending development of HAPE. This would seem to be strong evidence that essentially *all* normal people are subject to HAPE given sufficient hypoxia and exertion, and fits with the emerging notion that we should perhaps focus on what protects people from HAPE rather than what promotes susceptibility.

F. Effect of 36-48 hours at White Mt. on Exercise Responses

All of the preceding discussion is based on the sea level data obtained in San Diego. To that extent it may well reflect constitutional differences in exercise responses between HAPE-S and control subjects. However, one cannot rule out the unlikely possibility that short-lived episodes of HAPE months or years previously leave residual structural or functional changes in the lungs that continue to influence exercise responses.

Despite these sea level differences, 36-48 hours at White Mt. (3810m) led to convergence of the two groups' responses. The HAPE-S data were unaffected by White Mt. residence—rather, vascular pressures of the control subjects were higher at altitude. These data represent a snapshot in time during acclimatization, and it is difficult to fully interpret their meaning. Perhaps our control subjects have a delayed vascular response to hypoxia since the same degree of hypoxia at sea level applied for about 30 minutes did not produce this.

Of considerable interest is the observation that in both groups of subjects, the alveolar-arterial PO_2 difference was greater at White Mt. than at San Diego under each experimental condition. This was not due to more $\dot{V}\text{A}/\dot{Q}$ inequality at altitude, as the $\dot{V}\text{A}/\dot{Q}$ parameters were similar at both locations. Computed O_2 diffusing capacity of the lung was therefore reduced at White Mt., similarly in both groups. While this could reflect some interstitial edema, such an hypothesis would have to accept the fact that such edema failed to worsen $\dot{V}\text{A}/\dot{Q}$ inequality at the same time. Perhaps a more attractive hypothesis is that the diuresis of acute altitude exposure, caused by bicarbonate excretion to compensate the respiratory alkalosis, reduces capillary blood volume in the lung. This will reduce diffusing capacity due to its dependence on blood volume, yet need have no direct implications for $\dot{V}\text{A}/\dot{Q}$ inequality.

SUMMARY

Comparing the physiological responses of HAPE-S and control subjects to exercise in normoxia and hypoxia has revealed

1. Greater than normal vital capacity in control subjects.
2. Lower pulmonary vascular pressure responses to exercise in control subjects.
3. That PAOP is a significant contributor to elevated PAP in both groups of subjects during heavy exercise, and that the greater PAOP in HAPE-S subjects explains almost 75% of their greater PAP response.
4. That $\dot{V}\text{A}/\dot{Q}$ inequality increases with exercise, more so in HAPE-S than control subjects, and relates closely to pulmonary vascular pressures rather than the total cardiac output or ventilation.

Combining the present results with those from other studies in the literature, we propose that

1. HAPE is liable to occur in *all* subjects if ascent is too fast and exercise too vigorous.
2. Elevated pulmonary downstream vascular pressures likely are important in pathogenesis of HAPE.

3. Having a larger than normal lung volume may confer HAPE resistance, possibly by reducing pulmonary vascular resistance.

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CHAPTER 22

HYPOXIC VENTILATORY RESPONSE AND HYPOXIC PULMONARY VASCULAR RESPONSE IN HAPE- SUSCEPTIBLE SUBJECTS

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Introduction

Previous studies demonstrated that there are considerable interindividual differences regarding susceptibility to high altitude pulmonary edema (HAPE)¹. Furthermore it was shown that susceptibility to HAPE is associated with an increased hypoxic pulmonary vascular response (HPVR) during brief exposure to normobaric hypoxia at low altitude^{5,6,14,15} and with a decreased hypoxic ventilatory response (HVR) both during HAPE² as well as after recovery at low altitude⁸. There was, however, a considerable overlap of individual data between susceptible and non-susceptible individuals for both HPVR and HVR measurements in all studies.

Thus, by using either of these measurements it is not possible to reliably detect susceptibility to HAPE. We hypothesized that the identification of individuals prone to HAPE could be improved when HVR and HPVR measurements were combined. Since such testing for susceptibility is applicable for clinical use only when non-invasive measurements are involved, we estimated HPVR by Doppler echocardiography. For the same reason, levels of hypoxia were chosen that did not lead to an arterial oxygen saturation below 80% during steady state exposure. Tests under progressive hypoxia were terminated when this level for oxygen saturation had been reached.

We hoped to improve the power of discrimination by examining only subjects whose tolerance to high altitude had been established by means of clinical investigations and chest radiographs during identical exposures to high altitude. Therefore, we examined HVR and HPVR at low altitude in 30 subjects who had participated in previous high altitude studies carried out at the Capanna Regina Margherita between 1986 and 1990.

Methods

Study design

Thirty male subjects were recruited from participants in previous studies performed at the Capanna Regina Margherita (4559m) between 1986 and 1990. They had all ascended from 1170 to 4559m within 24 hours with an overnight stay at 3600m. Subjects were considered not to be susceptible to AMS when their AMS-C score was < 0.7 and the clinical score < 3 in all examinations at high altitude. Susceptibility to AMS was assumed when the average scores obtained at 4559m over two or three days were > 1.0 for AMS-C and > 3.0 for the clinical score. Subjects were considered susceptible to HAPE when they had at least 2 radiographically documented episodes of HAPE of which one had occurred during our high altitude studies.

Age, AMS scores and the number of radiographically documented episodes of HAPE are given in table 1. The subjects were studied in Heidelberg, Germany, at an elevation of about 100m. None of the subjects had stayed at altitudes above 2000m during the last two weeks or above 1000m during the last week before the study. The examiners did not know the subjects' susceptibility.

Table 1

Characteristics of the study groups

	Control	AMS	HAPE	p [§]
Subjects (n)	10	10	10	
Age (y)	40 (26-56)	39 (29-52)	41 (28-55)	0.83
History				
- AMS-C score	0.2 ± 0.07	1.7 ± 0.2**	1.58 ± 0.4**	< 0.001
- Clinical score	2.5 ± 0.2	5.8 ± 0.4**	5.4 ± 0.7**	< 0.001
- Episodes of HAPE	0	0	2.8 (2-4)	< 0.001

Scores are taken from preceding studies. Figures in parentheses are ranges. BMI = Body Mass Index. Values are means ± SE. p[§] refers to Kruskal-Wallis test, ** = p < 0.01 compared to the control group (Nemenyi test).

Measurements of hypoxic ventilatory response

The hypoxic ventilatory response (HVR) was measured by progressive hypoxia of 7 to 10 min as described in more detail elsewhere¹¹. Minute ventilation, end-tidal PO₂ and PCO₂ were measured by an open ergospirometric system based on the breath-by-breath analysis (Oxycon Beta, Mijnhardt, Bunnik, Netherlands). Arterial oxygen saturation (SaO₂) was continuously monitored by an oximeter using the finger probe (3740 pulse oximeter, Ohmeda Biox, Louisville, USA). Isocapnic HVR was measured by adding CO₂ to the inspired air during progressive hypoxia in order to maintain the end-tidal PCO₂ at the value observed during quiet breathing of room air. No CO₂ was added to the inspired air during progressive hypoxia for the measurement of

the poikilocapnic HVR. The hypoxic ventilatory response was analyzed by relating VE to SaO_2 . The relationship of VE to SaO_2 , which is considered to be linear²⁴, was analyzed by fitting data to a linear equation: $\text{VE} = b\text{SaO}_2 - \text{intercept}$, where $b = \Delta \text{VE}/\Delta \text{SaO}_2$. The SaO_2 values on the abscissa were scaled from high to low in order to obtain a positive slope.

Measurement of hypoxic pulmonary vascular response

Hypoxic pulmonary vascular response was assessed by Doppler echocardiography. From a reservoir bag, subjects breathed air at an FiO_2 of 0.21, 0.14 and 0.12. Each gas was breathed for a period of 10 min with 10 min rest on room air between each period. Doppler echocardiographic recordings were made in the 10th minute of each period. The order of the O_2 -concentrations was random and unknown by both subject and examiner of the echocardiographic study. Doppler recordings were performed using a conventional ultrasound machine (Aloka SSD-870, PPG Hellige) and 2.5 M Hz transducers. In order to estimate systolic pulmonary artery pressure peak flow velocities of tricuspid regurgitant jets were measured using continuous wave Doppler. The ratio of pulmonary-artery acceleration time to right ventricular systolic ejection time (AT/RVET) was used as another independent estimate of pulmonary-artery pressure. The recording obtained after 10 minutes at 21% O_2 was taken as baseline measurement. Data analysis was performed off-line independently by the second observer who did not know from which subject a specific recording had been obtained nor what O_2 -concentration was being breathed.

The right ventricular to right atrial pressure gradient (TP) was calculated from the maximum velocity within the tricuspid jet (V) of at least 3 beats using a modified Bernoulli equation: $\text{TP} = 4 \text{ V}^2$. In 22 examinations involving 9 subjects, pressure gradients could not be calculated because of insufficient Doppler spectra or absent tricuspid regurgitation.

Statistical analysis

Differences between groups were compared by non-parametric analysis of variance (Kruskal Wallis) and Nemenyi test for *post-hoc* testing. Two-sided p values are reported. Relationships between variables are examined by linear regression. Values are given as means \pm SE.

Results

Isocapnic HVR was significantly lower in the HAPE compared with the control group (Table 2) with only minimal overlap of individual values among these 2 groups. We found no value below 0.93 l/min/% in the control group while only 3 HAPE-S subjects had HVR values above 0.8 (between 1.0 and 1.05) l/min %. There was, however, a large scatter of individual values in the AMS group over the whole range covered by the control and HAPE group.

We found also a trend towards lower poikilocapnic HVR in the HAPE group which was statistically not significant (Table 2). The overlap of individual values among the control and HAPE group was considerably greater compared with the isocapnic HVR, while the AMS group showed again a wide scatter of individual values.

The pressure gradient at the tricuspid valve (TP) significantly increased with hypoxia in the control and HAPE groups. During the exposure to an FiO_2 of 0.12, TP

was significantly higher in the HAPE compared with the control group (Table 3). Individual values of all subjects were overlapping within a range of 17 to 33 torr with the exception of 3 individuals of the HAPE group showing TP values between 40 and 70 torr. It is noteworthy that TP could not be assessed in 9 subjects due to insufficient Doppler spectra or absent regurgitation.

Table 2

Acute hypoxic ventilatory response

	Control	AMS	HAPE	p [§]
Subjects (n)	10	10	10	
HVR isocapnic				
Δ VE/Δ SaO ₂ (l/min/%)	1.5 ± 0.2	1.2 ± 0.2	0.8 ± 0.1**	< 0.01
HVR poikilocapnic				
Δ VE/Δ SaO ₂ (l/min/%)	0.7 ± 0.1	0.6 ± 0.1	0.4 ± 0.1**	0.16

Values are means ± SE. p[§] refers to Kruskal-Wallis test, ** = p < 0.01 for the comparison of changes from the control group (Nemenyi test).

Although average values of AT/RVET mirror the significant changes observed for TP (Table 3), we found no significant correlation between the 2 parameters. Furthermore, an increase of AT/RVET under hypoxia in 5, 2 and 3 subjects of the AMS, control and HAPE group respectively, suggested a decrease of pulmonary artery pressure which is unlikely to have occurred. Thus, it appears that the changes of AT/RVET did not reflect changes in pulmonary artery pressure reliably.

Table 3Hypoxic pulmonary vascular response (FiO₂ 0.12)

	Control	AMS	HAPE	p
TP (mmHg)				
FiO ₂ 0.21	19 ± 2	19 ± 1	22 ± 1	0.15
FiO ₂ 0.12	29 ± 1*	23 ± 2	36 ± 6 ⁺⁺ , *	0.03
Subject (n)	6	7	8	
AT/RVET				
FiO ₂ 0.21	0.45 ± 0.02	0.40 ± 0.02	0.43 ± 0.02	0.25
FiO ₂ 0.12	0.42 ± 0.02*	0.44 ± 0.02	0.36 ± 0.02 ⁺⁺ , *	0.02
Subject (n)	10	10	10	

TP = pressure gradient at tricuspid valve. AT/RVET = acceleration time in pulmonary artery expressed as fraction of the right ventricular ejection time. Values are means ± SE. * p < 0.05 compared to the AMS group (Nemenyi test). * p < 0.05, ** p < 0.025 compared to values at FiO₂ = 0.21 of the same group (Wilcoxon signed rank test).

Discussion

This study demonstrates that HAPE-susceptible subjects have a low HVR compared to mountaineers with a good tolerance to high altitude. This finding is in agreement with previous studies^{2,8} and suggests that a greater overlap of individual values in the previous studies might in part be attributed to AMS-susceptible subjects in the respective control groups. Our data imply that a high isocapnic HVR ($> 1.1 \text{ l/min}/\%$) may rule out susceptibility to HAPE. This conclusion is, however, questioned by a recent study reporting very high HVR values in 2 of 4 HAPE-susceptible subjects¹². On the other hand, 2 previous investigations^{2,8} assessing HVR by methods comparable to the ones we used, found that all 17 HAPE-susceptibles had HVR values that were within the range obtained for the HAPE group of the present study.

Our data also demonstrate that susceptibility to AMS is not associated with a low HVR, confirming previous studies of Milledge^{9,10} who reported no correlation between HVR assessed at low altitude and the occurrence of AMS during expeditions. These observations are not necessarily in disagreement with studies reporting direct or indirect evidence of relative hypoventilation in AMS subjects compared with healthy controls at high altitude^{1,3,13} or during acute exposure in low-pressure chambers^{7,11}. It is possible that the HVR changes during exposure to high altitude, and that a fall of HVR and thus a lower ventilatory drive at high altitude is associated with the occurrence of AMS. We have recently observed some evidence supporting this hypothesis⁴. The association between a decrease of HVR during exposure to high altitude and symptoms of AMS was, however, rather weak.

Estimations of pulmonary artery pressure under hypoxia by Doppler echocardiography showed a significantly higher mean increase of pulmonary artery pressure in HAPE vs. AMS susceptibles but not vs. control subjects. Except for 3 individuals with an exaggerated HPVR it was, however, not possible to discriminate between AMS and HAPE-susceptible individuals with low HVR values by non-invasive estimations of pulmonary artery pressure under hypoxia. The lack of further discrimination may in part be attributed to the limitations of Doppler echocardiography for accurately detecting small changes of pulmonary artery pressure in the order of 5 to 15 torr as they have been observed after brief hypoxic challenge in studies measuring pulmonary pressure by catheterization^{6,14,15}. Further possible explanations for our failure to confirm the hypothesis of this study are 1) that a short exposure of 10 min to an FiO_2 of 0.12 may not give rise to the full HPVR which occurs during exposure to high altitude and 2) that additional, not examined factors are important for the pathogenesis of HAPE.

Conclusions

- 1) Subjects with a low HVR and an exaggerated HPVR have a high risk of developing HAPE.
- 2) Susceptibility to AMS and HAPE cannot be reliably identified at sea level by measurements of HVR and Doppler echocardiographic estimation of HPVR.
- 3) There is no relation between the HVR determined at sea level and susceptibility to AMS.

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CHAPTER 23

MICRONEUROGRAPHY IN HAPE

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Introduction

The sympathetic nervous system plays a key role in the regulation of cardiac output, arterial pressure and regional blood flow. Hypoxemia, such as that resulting from exposure to high altitude, induces a variety of cardiovascular adjustments which are, at least in part, mediated by the sympathetic nervous system. For example, there is evidence in both anesthetized and conscious animals, that hypoxia is a potent stimulus to the sympathetic nervous system as determined by measurements of plasma catecholamines or direct nerve recordings^{3,4,14,15}. Such sympathetic activation is important because it is accompanied by increases in regional vascular resistance.

In contrast to these studies in experimental animal models, studies in humans examining effects of hypoxia on the sympathetic nervous system have produced conflicting results. For example several studies using plasma norepinephrine concentrations as a marker of sympathetic activity did not find any detectable increase during acute hypoxia. Furthermore, studies using measurements of regional vascular resistance as an indirect index of sympathetic activity in humans also failed to demonstrate an increase during acute hypoxia.

Similarly, during acute exposure of humans to high altitude plasma norepinephrine levels have not consistently been found elevated¹¹. Taken together, these findings based on plasma norepinephrine measurements or indirect indices of sympathetic activity provide no evidence for sympathetic activation in humans either during acute hypoxia under laboratory conditions or after acute exposure to high altitude.

One of the limitations of these studies is, however, that they used either indirect indices (regional vascular resistance) or measurements of plasma norepinephrine concentrations to assess effects of hypoxia on sympathetic activity. While under many conditions there is a close correlation between changes in sympathetic nerve activity (as determined by direct intraneuronal measurements) and corresponding changes in regional vascular resistance²³, there are other conditions where changes in sympathetic activity and regional vascular resistance are dissociated. For example, during insulin infusion in lean healthy humans there is now abundant evidence that insulin evokes both marked sympathetic activation and vasodilation in the same vascular bed (i.e. skeletal muscle)^{1,25}. The latter has recently been shown to be related to insulin-induced stimulation of nitric oxide (NO) release, because it is abolished after infusion of N^G-monomethyl-L-arginine (L-NMMA), an analogue of L-arginine that acts as a

competitive stereospecific inhibitor of NO-synthase¹⁹. While the exact underlying mechanism by which insulin stimulates NO release remains to be determined, recent evidence indicates that there exist non-adrenergic non-cholinergic sympathetic nerves. This suggests the possibility that insulin-induced vasodilation in skeletal muscle could be related to stimulation of sympathetic vasodilatory nerves (and thereby NO release), and offers one potential explanation for the seemingly paradoxical observation of insulin-induced stimulation of sympathetic ("vasoconstrictor") outflow and blood flow to the same vascular bed.

With regard to plasma norepinephrine concentration measurements, it is now well established that plasma norepinephrine concentrations not only depend on plasma norepinephrine spillover from sympathetic nerve endings but also on other factors such as neuronal reuptake and clearance. Interestingly, a recent study suggests that in humans, hypoxia increases plasma norepinephrine clearance¹². This observation may suggest that the lack of an increase in plasma norepinephrine levels observed during acute hypoxia could be related, at least in part to enhanced removal.

To overcome some of the limitations of these traditional methods to study sympathetic function in humans, microneurography has become the focus of much interest over the past few years. This technique which has been developed by Swedish neurophysiologists²⁴ allows us to directly record postganglionic sympathetic nerve action potentials in conscious humans. This technique has several distinctive advantages over the more traditional methods. Among the most important are: a) this technique allows us to examine directly and specifically the reflex neural regulation of a vascular bed (i.e. skeletal muscle); b) this neural activity responds very rapidly, i.e. within one or two cardiac cycles, to reflex maneuvers (thus the timing of neural as compared to hemodynamic events can provide important clues regarding cause-and-effect-relationships); and c) the neural activity provides considerable complementary information to that provided by measuring plasma catecholamines. For example, by recording muscle sympathetic nerve activity (MSNA), one can determine if increases in plasma norepinephrine are due in part to increased central sympathetic outflow. Under certain conditions, but not in others, there exists a general correlation between venous plasma norepinephrine levels and MSNA. We generally have found that when such a correlation exists, plasma norepinephrine is a very insensitive measure of MSNA (as it seems to take a 500% increase in the neural activity to cause a barely detectable increase in plasma norepinephrine). Furthermore, and of potential importance for the use of microneurography to examine effects of high altitude under real life conditions, in any given subject the rate of sympathetic nerve firing is remarkably stable and varies by <15% from one experimental session to the next.

Recently several studies have employed microneurography to examine effects of acute short-lasting hypoxia on MSNA^{16,17,21}. In these studies, there seemed to exist quite a great interindividual variation in responses, but in general, mild hypoxia ($\text{FIO}_2 \geq 12\%$) had little or no effect on MSNA, whereas more severe hypoxia ($\text{FIO}_2 \leq 10\%$) evoked marked increases in MSNA. Interestingly, increases in MSNA were not accompanied by concomitant increases in plasma norepinephrine concentrations. While these studies suggest that in accordance with previous observations in experimental animals, hypoxia stimulates sympathetic activity in humans, no microneurographic data have been obtained at high altitude, and no attempt has been made to compare MSNA responses to hypoxia between HAPE-susceptible and HAPE-resistant subjects.

Investigating sympathetic function in such patients may be important however. Indeed, hypoxic pulmonary vasoconstriction is a hallmark of HAPE^{2,5,6,8,9} and is thought to play a key role in its pathogenesis^{6,9,20,22}. While the exact underlying mechanism causing exaggerated hypoxia-induced pulmonary hypertension in HAPE remains unknown, preliminary data in humans suggest that sympathetic activation could contribute to pulmonary hypertension in HAPE⁷. Indeed, in subjects with HAPE, the alpha-adrenergic blocking agent phentolamine was found to be markedly more potent in lowering pulmonary artery pressure than other non-specific vasodilators.

In the following, we will briefly describe preliminary microneurographic data obtained in HAPE-resistant and HAPE-susceptible mountaineers studied both during acute hypoxia at low altitude, and at high altitude.

We studied mountaineers who had a history of radiographically documented HAPE within the previous 4 years (HAPE-prone subjects). Mountaineers with a history of repeated alpine style climbing to peaks above 4000m but no symptoms of HAPE or acute mountain sickness (HAPE-resistant subjects) served as controls. Effects of acute hypoxic breathing on MSNA were tested at low altitude, and tests examining effects of exposure to high altitude were done 18 or 24 h after arrival at the Capanna Regina Margherita (4559m). To test effects of acute hypoxia at low altitude, subjects breathed four gas mixtures sequentially for 12 min each; 1) FI O₂ = 0.21, control; 2) FI O₂ = 0.14, 3) FI O₂ = 0.12, and 4) FI O₂ = 0.10. To assess potential functional consequences of hypoxia-induced sympathetic activation we also measured systolic pulmonary artery pressure using Doppler echocardiography.

Recordings of Sympathetic Nerve Activity

Multiunit recordings of postganglionic sympathetic nerve activity were obtained with unipolar tungsten microelectrodes inserted selectively into muscle nerve fascicles of the peroneal nerve posterior to the fibular head by the microneurographic technique of Vallbo *et al.*²⁴. The neural signals were amplified 20,000 to 50,000 times, filtered (bandwidth 700-2000 Hz), rectified and integrated (time constant 0.1 s) to obtain a mean voltage display of sympathetic activity. A recording of sympathetic activity was considered acceptable when it revealed spontaneous, pulse synchronous bursts of neural activity, with the largest bursts showing a minimal signal to noise ratio of 3:1. In each study, we documented that we were recording sympathetic outflow to skeletal muscle by demonstrating that the neural activity did not respond to arousal stimuli (loud noise) or skin pinch, but showed a characteristic biphasic response to the Valsalva maneuver¹⁸.

Findings during acute hypoxia at low altitude

We found that during hypoxic breathing at low altitude in HAPE-prone subjects, increases in MSNA started earlier and were 2-3 times larger than those observed in HAPE-resistant subjects. Moreover, such exaggerated sympathetic activation was accompanied by augmented hypoxic pulmonary vasoconstriction.

Findings at high altitude

At high altitude technically satisfactory recordings were extremely difficult to obtain. However, we succeeded in several subjects. Our data show that compared to the resting rate of sympathetic nerve discharge recorded at low altitude, all subjects

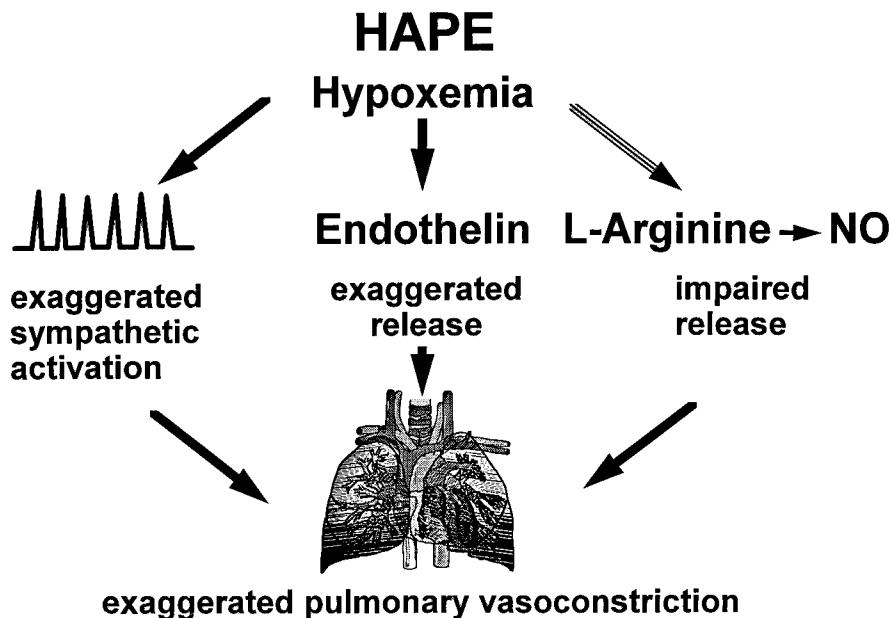


Figure 1 Factors that may contribute to exaggerated hypoxic pulmonary vasoconstriction in HAPE.

had markedly augmented rates of sympathetic nerve firing 24 to 36 h after arrival at the Capanna Regina Margherita. Moreover, and consistent with our preliminary findings at low altitude, increases in MSNA burst frequency between low and high altitude tended to be larger in HAPE-prone than in HAPE-resistant subjects. It is of note here that at the time of the study none of the HAPE-prone subjects had radiographic evidence of HAPE. However, several of these subjects developed HAPE within the next 24 hours following microneurography. Our findings suggest therefore that in some subjects, HAPE is preceded by dramatic increases in the rate of sympathetic nerve discharge.

Taken together the present data demonstrate the feasibility of microneurography under extreme conditions (for both the subject and the investigator) in the setting of a high altitude laboratory. These preliminary results provide evidence for an augmented hypoxia-induced sympathetic activation in HAPE-prone subjects. We speculate that such sympathetic overactivity could be one of the factors that contribute to exaggerated pulmonary vasoconstriction at high altitude and in turn may play an important role in the pathogenesis of HAPE. Other factors which, in concert with exaggerated sympathetic activation, could contribute to augmented pulmonary hypertension in HAPE (Fig. 1) are the recently described impairment of NO release (Vollenweider *et al.*, unpublished observations) in such patients, as well as augmented stimulation of endothelin release (Scherrer *et al.*, unpublished observations). Further studies are needed to delineate the relative contributions of these three potential pathogenetic factors.

In conclusion, we have provided evidence that microneurography is ready to go up to the mountains. Future studies employing this powerful tool to examine sympathetic function in humans should provide important new insight into underlying mechanisms of the regulation of the sympathetic nervous system at high altitude and its potential role in the pathogenesis of HAPE.

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CHAPTER 24

DOES HYPOBARIA PLAY A ROLE IN THE DEVELOPMENT OF THE HIGH ALTITUDE ILLNESSES

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Introduction

The classic interpretation of hypoxia as the cause of acute mountain sickness (AMS) has seldom been questioned in this century. The altitude sickness-hypoxia paradigm was recently and clearly stated by Pierre DeJours, a prominent respiratory physiologist: “*it remains that the main factor of high-altitude illness is the hypoxia caused by the low barometric pressure, since a quick recovery is obtained by inhalation of an oxygen-enriched mixture...*”⁴. In this paper we review some concepts regarding the effects of hypobaria *per se* on physiological responses to high altitude in an attempt to broaden our understanding of the complicated pathophysiology of the high altitude illnesses. In particular, we will focus the discussion on the pathophysiology of AMS.

Any discussion of experiments that investigate the pathophysiology of AMS must begin with a description of AMS and a definition of its measurement. Only recently has an international standard existed for the definition and measurement of the symptoms of AMS. Previously, many different systems, from purely clinical descriptions to a wide variety of questionnaire and interview techniques have been used. One major problem with a historical analysis of factors which may contribute to AMS is a confusion of the symptoms of acute hypoxia with those of AMS. The recent Lake Louise Consensus AMS Scoring System states that a constellation of symptoms (headache, nausea, dizziness, fatigue and trouble sleeping) will only be called AMS when the victim has been at altitude for at least 2 hours²⁰. This limitation of the classification of AMS only in persons at altitude for 2 or more hours discriminates between the symptoms of acute hypoxia and AMS. A human exposed rapidly to the altitude of the summit of Mount Everest would lose consciousness within five minutes and possibly die within ten to twenty minutes. They would perish from lack of oxygen in the brain, not from AMS. Acute mountain sickness takes at least several hours to develop.

Does hypoxia alone cause AMS?

In the late 1800's, Bert was decompressed to 15,770 feet (4810 meters) until he experienced nausea and tachycardia.³ He then inhaled oxygen and noted that each breath of oxygen relieved symptoms and tachycardia, which returned after inhalation of oxygen was stopped. These symptoms are clearly due to acute hypoxia, and although prolonged exposure may have caused the classic syndrome of AMS, in itself these experiments do not, in our opinion, prove a causal relationship between hypoxemia and the development of AMS. Barcroft's "Glass House" experiment in 1920 is often quoted as proof that hypoxia is the cause of AMS¹. In this experiment Barcroft was exposed to decreased inspired oxygen at constant sea level barometric pressure. On the sixth day, after gradual ascent he reached an "altitude" of 18,000 feet (5486 meters). He awoke with a headache, vomiting and had "difficulty of vision." Was this AMS? Haldane, a contemporary of Barcroft, was skeptical; he wrote in 1925 that Barcroft "became extremely ill and his body temperature had risen"¹¹. Fever is not usually associated with AMS. Furthermore, Barcroft reports¹ that he reliably became ill within hours after arrival at mountain altitudes as low as 10,000 feet (3048 meters).

Several experiments have indirectly examined normobaric hypoxia and AMS. Meehan and colleagues exposed 7 men to 6 hours of mild exercise with an inspired oxygen of 12.5%¹⁶. None of the subjects developed AMS in spite of arterial PO₂ values of 42±3 mm Hg. Swenson and co-workers exposed 12 subjects to 12% inspired oxygen for 6 hours and none experienced AMS²³. In another study, Tucker *et al.* exposed 6 subjects to 2 hours of simulated altitude (hypobaric hypoxia) and on a separate occasion to 2 hours of normobaric hypoxia. Mean symptom scores for the altitude exposure were 8.3±1.6, compared to 3.2±1.2 during normobaric hypoxia²⁴. In only one study published to date have symptoms of AMS been reported due to normobaric hypoxia. Knight and colleagues completed an interesting study to examine the symptom responses to flame retardant atmospheres designed to mimic the environment encountered when living aboard submarines.¹² They exposed 12 men to 17% and 13% oxygen for 63 hours. Some AMS symptoms were present with 13% oxygen during the last few hours of the exposure; details of individual symptom severity and comparison with altitude were not given. Knight *et al.* concluded that the design of their experiment precluded "drawing conclusions about the equivalency of symptoms between hypobaric and normobaric hypoxic at the same low oxygen level."

Our recent study supports the notion that there are differences in the AMS symptom response profiles between normobaric and hypobaric hypoxia. Our preliminary data suggested that normobaric hypoxia did not cause the symptoms of AMS; hypobaria seemed somehow necessary¹⁴. Study of additional subjects taught us a slightly different lesson. During hypobaric hypoxia the symptoms of AMS occurred earlier and were more severe than during normobaric hypoxia, in spite of nearly identical inspired PO₂'s. This leads us to consider the possibility that hypobaria may exert specific effects on human responses to high altitude.

Evidence of a role for hypobaria in the pathophysiology of the high altitude illnesses.

We will focus this discussion on several physiological responses to hypobaria that indicate a need to carefully consider the role of hypobaria in the pathophysiology of the high altitude illnesses. In 1988, Levine and the Matsumoto group published a study of the lymph flow in sheep exposed to increasing degrees of hypoxia or

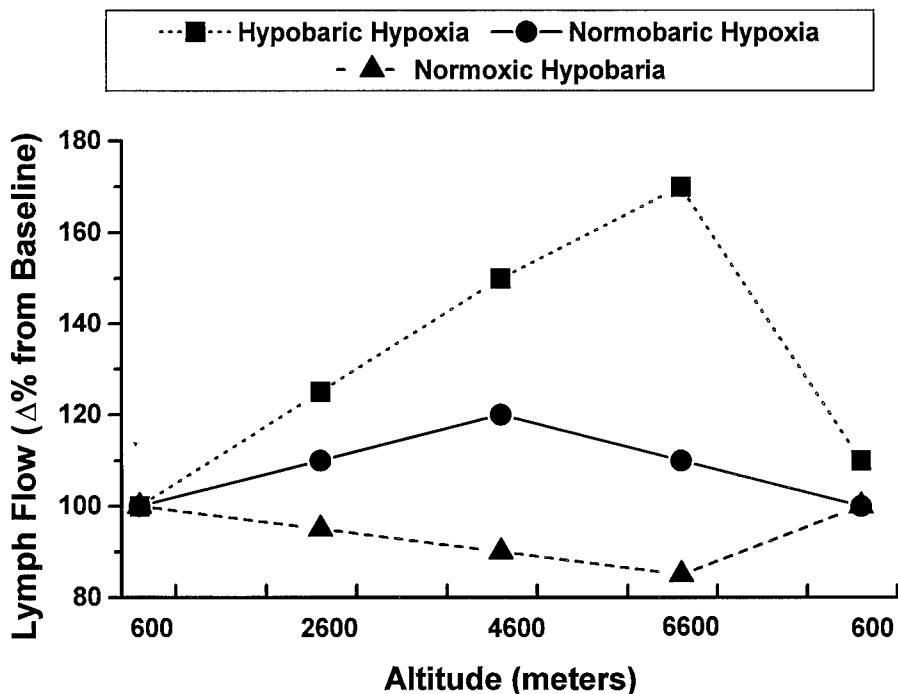


Figure 1 Lung lymph flow in sheep exposed to different combinations of hypobaric hypoxia, normobaric hypoxia and normoxic hypobaria. The percent change in lung lymph flow during the hypobaric hypoxia (altitude) trial was not simply the sum of the effects of hypoxia and hypobaria, suggesting a unique synergism. Adapted from reference 13.

hypobaria.¹³ The experiments were done in hypoxia, hypobaric hypoxia, or normoxic hypobaria. The hypobaria matched the decreased pressures attained in the hypobaric hypoxia trial. What has intrigued physiologists since this paper first appeared is that the increased lung lymph noted during hypobaric hypoxia was not just the sum of the effects during normobaric hypoxia and normoxic hypobaria, but that the results suggested that there was a synergy between the effects of the normobaric hypoxia and normoxic hypobaria (Fig. 1). This effect remains unexplained.

In the late 1960's considerable research was conducted on the physiological effects of normoxic and hyperoxic hypobaric environments because of interest in altering environments in space exploration vehicles. In one of these studies Epstein and Saruta made the unexpected discovery that prolonged hypobaria (258 mm Hg) caused a marked decrease in creatinine clearance (see Figure 2), a tendency to sodium retention and a smaller decrement in body weight than in controls.⁵ They studied eight male subjects during 7 days of hypobaria or control after the subjects reached steady sodium and fluid balance. After the 7th day of hypobaria creatinine clearance had decreased 40%. The authors concluded that "*the isolated variable of reduced barometric pressure produces a positive cumulative water balance...*". If hypobaria alone can cause perturbations of the body's mechanisms for fluid balance the implications for hypobaria in the pathogenesis of high altitude illnesses becomes more direct. Many authors have hypothesized that an important shared feature of all high altitude illnesses is fluid retention.^{2,9,22}

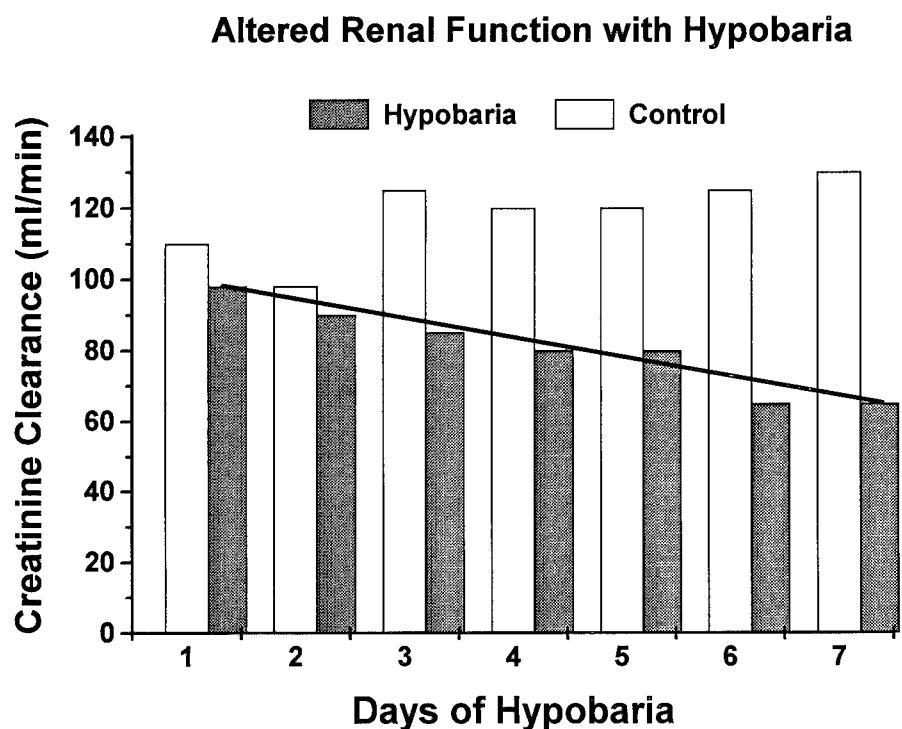


Figure 2 Creatinine clearance decreased 40% in normal healthy human male volunteers exposed to 7 days of normoxic hypobaria ($P_b=258$ mm Hg) compared to the control condition. Adapted from reference 5.

In addition to fluid retention, the adequacy of resting ventilation for the hypoxic stress of high altitude is another of the main factors recognized as contributing to the pathogenesis of high altitude illnesses.^{10,18} Does hypobaria have any demonstrable effects on resting minute ventilation? The first evidence that hypobaria may have a significant effect on ventilation came from a study published in 1983 by Tucker *et al.* at the University of Colorado.^{7,24} In this study nine healthy young men were exposed to hypobaric hypoxia and normobaric hypoxia during two separate trials. During the hypobaric hypoxia trial, resting minute ventilation increased about 30% above control values. During the normobaric hypoxia trial resting minute ventilation increased about 60% above control values, or a nearly 30% greater ventilatory response to normobaric hypoxia compared with hypobaric hypoxia. (Fig. 3) An explanation for this apparent blunting of ventilation during exposure to hypobaric hypoxia is not immediately apparent. In the discussion of these results the authors candidly describe their own reactions to these unexpected results: "Although intriguing, these observations have not been published before because we are at a loss to explain the mechanism responsible." We recently found a similar degree of blunting of resting minute ventilation by hypobaria when comparing hypobaric hypoxia and normobaric hypoxia.^{14,21} Recent unpublished observations on 9 subjects demonstrated a significantly higher (23%) ventilation after 6 hours of normobaric hypoxia than during simulated altitude at the same P_1O_2 of 81 mmHg.

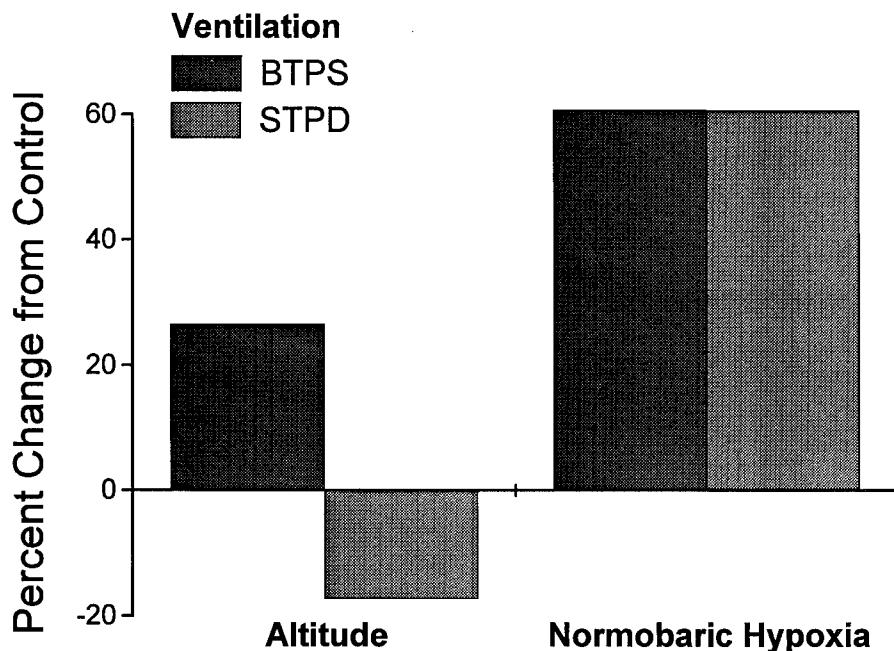


Figure 3 Resting minute ventilation was 30% less during exposure to hypobaric hypoxia (altitude) compared with normobaric hypoxia in 6 normal, healthy male subjects. Adapted from references 7 and 24.

Speculations on Possible Mechanisms for Hypobaria's Physiological Effects

As pointed out by Gray in his thorough review of the pathophysiology of HAPE, decompression to altitude is analogous to ascent from a saturation air dive, in which small decompression stages can produce intravascular bubble formation.⁶ Gray cites the work of Guillerm *et al.*⁸ to support his theory that bubble formation may be the mechanism by which hypobaria exerts its physiological effects. Guillerm *et al.* showed bubbles in the pulmonary arteries of miniature swine on ascent from sea level to only 2000 meters. In humans, they reported intravascular bubbles recorded precordially with rapid exposure to 4000 meters⁸. Recent mathematical simulations of bubble formation during hypobaric decompressions support the limited animal and human evidence and point to an interesting and perhaps important new area of investigation²⁵.

These studies suggest that in our recent study the 30% reduction in atmospheric pressure experienced at simulated altitude as compared to ambient pressure could cause the formation of microbubbles in the circulation. Such a temporal lag in liquid phase with gas phase equilibration could conceivably alter pulmonary and tissue gas exchange and vascular membrane permeability⁷. It is generally presumed that a pressure effect will not be seen with these small changes because it takes pressure changes an order of magnitude greater to induce readily observed physiological changes, such as those occurring with diving. However, this assumes a linear scale for cause-and-effect which may be an invalid assumption. For example, if one assumed a semi-log scale between cause (log x) and effect (y) then the response to a reduction in pressure from 3 to 2 atmospheres could be similar to that experienced in going from 6,000 to 15,000 ft (1830 to 4570 meters).

Another difference between altitude and normobaric hypoxia is the partial pressure of nitrogen (N_2). For example, when P_1O_2 is matched during normobaric hypoxia and hypobaric hypoxia, the P_1N_2 is 60% greater during normobaria. Evidence is available that increased amounts of N_2 ameliorate the effects of chronic pulmonary O_2 toxicity. This effect has been demonstrated at less than 2 atmospheres by enhanced survival time of mice¹⁹. Whether this alteration is due to influences on free radical formation or anti-oxidants is unknown.

Another consideration pertains to the density of the ventilated air. This alters the relationship between the work of breathing and airflow turbulence which will alter intrathoracic pressure. Empirically, the maximum breathing capacity will be increased about 20% for a 30% reduction in air density and reduced about 40% at 3.0 atmospheres¹⁵. If air density was responsible for a slight curtailment in ventilation during normobaric hypoxia, then the reduced density during hypobaric hypoxia would lead to increased ventilation rather than the observed blunted ventilation during hypobaric compared with normobaric hypoxia. Prolonged exposure to 3.0 atmospheres has been recently shown to result in a diuresis¹⁷, but whether the opposite tendency (fluid retention at decreased pressure) is true cannot be definitively answered at this time.

Summary

The symptoms of AMS are less severe and occur later during normobaric hypoxia compared with hypobaric hypoxia (altitude), even though inspired PO_2 is similar. We have reviewed support for the idea that fluid balance can be altered and ventilatory chemosensitivity reduced by hypobaria *per se* and that perturbations in these systems are linked to the pathophysiology of AMS. And finally, we offer microbubble formation as a possible mode of action for hypobaria in the pathophysiology of high altitude illnesses.

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CHAPTER 25

HIGH ALTITUDE INTERMITTENT CHRONIC EXPOSURE: ANDEAN MINERS

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INTRODUCTION

Most of the larger mines in the world located above 3,000m above sea level are in South America: Peru, Bolivia and Chile. In the Chilean Andes, copper, gold, silver and non-metallic minerals are exploited from open pits or underground deposits. These mines operate with a headcount ranging from 20 to 2,000 workers. The larger companies are owned by multinational companies such as BHP, RTZ, Outokumpu, Phelps Dodge, Cyprus, Angloamerican, Barrick, Exxon, etc., with head offices in USA, Canada, South Africa and Finland. ENAMI, CODELCO, Minera Pudahuel and others companies are the leaders of the national mining industry in Chile.

In 1994, there were about 12,000 workers above 3,000m carrying out mining jobs such as exploration, construction, extraction, grinding, concentration and shipping of ore as well as the operation and maintenance of solvent extraction and electrowinning plants, and transport, security, medical care and catering services. By year 2,000 there will be about 20,000 miners working above 3,000m. These companies use state-of-the-art technology with automatic, servocontrolled, computerised processes, laser and optic fiber, remote telecommands, high tonnage heavy machinery such as large trucks, shovels, drills, and earth moving equipment which require highly skilled personnel who are hired in the urban zones of Chile, all of them located below 1,000m.

Intermittent Chronic Exposure

The shift system depends on the distance between the city and the mine. In the mines located near a city, less than 1.30 hrs by bus, workers, technicians and professionals, not native to altitude, daily go from 0-800m up to 3,500-4,500m to work in 8-hour rotating shifts, i.e., morning, afternoon and night shifts, with 2-3 rest days at low altitude. In the mines located far from a city, more than 2h trips, the work day ranges from 10 to 12h per shift with 4 or more days at high altitude (HA) camps. Then they have 4 or more rest days at low altitude where their families live. The current shift cycles are: 8x4, 5x2, 4x3, 7x7, 14x7, 10x10 and 21x7 days depending on the distance from the mine to the place of residence.

The hypobaric hypoxia to which our workers are exposed has two elements: one acute element at the beginning of their regular shift in the mine, every 1 or 20 days, and a chronic one due to the repeated ascents for many years. We define this as Intermittent Chronic Exposure (ICE) to hypobaric hypoxia of miners.

Mining companies reactions

Operating mine costs are affected by altitude, specifically because of lower labor productivity, greater power requirements for some machinery and processes, heating necessitated by low ambient temperatures and the difficulty of achieving normal operating capacity in extreme weather conditions.

Table 1
Equipment Size Increases to Achieve Sea Level Output (%) at 3,000-4,000m.

Equipment	Output Unit	% Increases at altitude	
		3,000m	4,000m
Diesel engines	b.h.p.	40	55
Compressors	airtool work	55	75
Vacuum filters	tons/hr solids	30	45
Vacuum pumps	intake volume	30	40
Transmission lines	MVA - km	20	30
Transformers	MVA	15	25
Electrical machines	kw	15	25
Flotation	tons/hr	35	50
Leach vessels	tons/hr	50	85

Data taken from Edge⁵

At high altitude, mining machinery and equipment have to increase their size, power and protective elements if the same efficiency they have at sea level is to be attained⁵ (Table 1). At 3,000m the increase percentage fluctuates from 15% for transformers up to 55% for compressors. At more than 4,000m a 25% increase is required for electrical machines, and up to 85% in the case of leach vessels. At mine sites which have to endure cold climates, heavy snowfall and high wind velocities, typical in the Andes, building design has to be carefully considered: structural design, insulation, heating. Furthermore, consideration also has to be given to avalanche and earthquake risks. Likewise, the companies are aware that in order to achieve the physical work performance of workers like that obtained at sea level it will be necessary to increase the man/hour ratio. At 4000m 50-80% should be needed for those not native to altitude. The effects of HA and its accompanying meteorological conditions at a typical mine and beneficiation plant implies higher capital costs per unit of productivity capacity.

With respect to HA effects on workers the mining companies have been shown different ways of dealing with the altitude problem. Currently 3 kinds of company reactions prevail:

1. The HA problem does not exist and each worker decides to accept the work or not.
2. HA causes medical problems which must be treated as a common disease.
3. HA involves productivity, safety and health risks that can be controlled.

To select personnel, type 1 and 2 companies do not take into account the altitude problem, whereas type 3 companies are concerned with high altitude tolerance, be it through an ascent test or asking about previous exposures (Table 2). As far as AMS symptoms and related morbidity of visitors and workers are concerned, type 1 companies treat the problem with herbal tea, rest and descent, type 2 companies prescribe pharmaceutical products, oxygen and refer the critical cases to a general practitioner, and type 3 companies have a protocol for visitors and for lowlanders which includes preventive treatment with acetazolamide, AMS evaluation by means of a questionnaire, and scores and sends problem cases to doctors who are experienced in mountain medicine.

Table 2.
Systems for High Altitude Work of Mining Companies

	<i>TYPE 1 Co.</i>	<i>TYPE 2 Co.</i>	<i>TYPE 3 Co.</i>
1. Selection of personnel	-No	-No	-Yes
2. Treatment of AMS and morbidity related to HA	-folkloric traditional	-curative medicine	-mountain medicine
3. Measures reducing the impact on productivity	-economic incentive	-economic incentive	-to improve life quality at camp -oxygenation of critical places ?
4. Camp quality	-according to the company's standard or -agreements with trade union		-to minimize HA's effects and -extend contractual relationship

The type 1 or 2 companies that detect workers' lower productivity at high altitude use the economic incentive to solve the problem. Type 3 companies envisage improvement of the quality of life at sites and camps, and probably consider adding oxygen in bedrooms. The camp quality of companies 1 and 2 is subject to their own common standards or to agreements with trade unions. On the other hand, type 3 companies install good humidification and heating systems, cafeterias and gymnasiums in the camps and radiotelephonic communication with the workers' families to compensate for the adverse geographic and climatic conditions.

The concern of type 3 companies to minimise HA effects on workers has the following final objective:

- (i) To prevent early resignation of newly hired personnel, which in type 1 and 2 companies amounts to 5-10% in the first shifts.
- (ii) To maximize work productivity and safe conduct at critical positions and during operation of high cost machinery.
- (iii) To ensure a long-lasting contractual relationship.

The modern mining companies over 3,000m with ICE system try to establish policies and procedures which will maximize employee efficiency consistent with appropriate health and safety standards. To achieve this, different biomedical answers are required for different subjects (Table 3).

Table 3
High Altitude Mining Work and Health: Priorities for the next decade.

Personnel selection system
Control of acclimatization period of the newly hired employees
Control of Productive Performance and Heavy Work: cognitive and physical
Safety and Industrial Hygiene risks
Medical risks, chronic and acute morbidity, and aging.
Mental health and life quality at Camp
Biological Cycles: Sleep and Shift System
Employment Benefits
Medical Assistance System at Camp

From Jimenez, 1994¹¹

Intermittent Chronic Exposure Studies

In order to resolve problems of HA Intermittent Chronic Exposure under working requirements there is little biomedical information and all this, has been recently published^{4,2,10,9,7,6,3,18,11}. One of the main drawbacks for companies located over 4,000m is to get sufficiently skilled personnel. They have to compete against mines at lower altitude and one of the ways of doing this is to offer better working conditions, camps and benefits. Compañía Minera Collahuasi is in the aforementioned situation. It will install mining operations, on the Chilean Andean altiplano, with more than 1,600 workers at 4,200 and 4,600m. This is why since 1993 the company has been supporting multidisciplinary altitude studies of the miners' intermittent chronic exposure to find preventive and control strategies that may enable the degree of health, work and quality of life to be optimised. The Collahuasi studies have been made by researchers belonging to Chilean Universities and health occupation centers, having as advisers Dr. C. Monge, J.B. West and J-P. Richalet. The population studied is 122 ICE acclimatized miners, in copper companies over 4,000m, with 7x7, 10x10 and 10x7 days shifts. The following are some of the results.

The Table 4 shows the characteristics of 66, 37-year-old Collahuasi workers acclimatized to intermittent chronic exposure for 50 months on average. 77% of them are exposed to silicogenous dust and 68% to occupational noise. Even though it is a middle age group there is a low percentage of hypertensive individuals; obesity amounts to 7.5%, diabetes to 3%, and there is a high degree of nicotinism. The average level of cholesterol is normal with only 4.5% of hypercholesterolemia. The high HDL percentage stands out, lower than 45 milligrams, which increases the number of cases of cholesterol/HDL level which is more than 5 to 33.3%. There is also a high percentage of cases of hyperuric acid level, 10.6%, also found in other mining populations. Generally speaking, these factors show that the workers have transferred their urban life style to altitude. 3% of the workers from endemic zones have positive Chagas serology with normal electrocardiographic study.

Table 4
Risk Factors in 66 ICE Collahuasi's Workers 1994.
 Mean age: 37 years. Mean months at HA: 50 (3-178)

Risk Factors	Reference Value	Prevalence	
		n	%
Dust (silica)		51	77
Noise exposure		30	68
Diabetes		2	3.0
Hypertension	>140/90	2	3.0
Obesity	bmi>27.8*	5	7.6
Tobacco	current smoker	28	42.4
Cholesterol	mean 203 +/-34 mg/dl		
Cholesterol high	=>240 mg/dl**	3	4.5
HDL low	<45 mg/dl	50	75.7
Cholesterol/HDL	>5	22	33.3
Uric acid high	=>7.5 mg/ml	7	10.6
Urine test (+)	proteinuria, hb	1	1.5
Chagas serology (+)		2	3.0

* From Healthy People⁸. **From The Expert Panel²⁰

Table 5
Mean Hemoglobin According to Age Groups Between 3800 and 4600m
Intermittent Exposure Workers and Permanent Miners.

Exposure:	Intermittent	Intermittent	Permanent
Altitude, m:	4200-4600	3800-4100	4300
Shift, days:	10x10, 7x7	4x4, 8x4, 5x2	
Mine:	Collahuasi	El Indio*	Cerro Pasco**
	Hemoglobin	HB	HB
Age Groups	Mean se	Mean	Mean se
TOTAL	16.7±1.5	17.4	18.9±1.6
20-29	16.3±1.2	17.5	18.3±1.5
30-39	16.6±1.6	17.7	18.8±1.8
40-49	17.2±1.5	17.6	19.0±1.9
50 y más	16.2±1.0	17.4	19.3±2.2

* From Cantuarias J, 1994¹⁵. ** From Monge C *et al*, 1989¹⁶.

The average hemoglobin level at Collahuasi is 16.7g, lower than the 18.9g that permanent miners at the same altitude in Cerro Pasco have¹⁶ (Table 5). Miners with intermittent exposure to a lower altitude, like those in El Indio, have a slightly higher hemoglobin level: 17.4 grammes¹. The difference might be due to the different duration of the periodic stays at sea level, 2-4 days El Indio workers and 7-10 days Collahuasi miners.

The quality of sleep of workers acclimatised by ICE was studied by a group of neurophysiologists of the University of Chile by means of the Spiegel test¹⁹. For each miner six pathological indexes were evaluated at sea level during their 1st and 5th rest day and their 1st and 5th day in the camp (Table 6). The pathologic indexes are: 1. to fall asleep within more 30 minutes, 2. to sleep a total of less than 5 hours, 3. to wake up 3 or more times in their sleep, 4. to wake up early involuntarily, 5. to have the feeling of not having rested after sleeping and, 6. to have a poor quality of sleep. The information gathered show that all the pathological indexes increase on the first day at high altitude. The difficulty of falling asleep increases by 8.3% at sea level and up to 45% at high altitude. At sea level, 27% of the workers sleep less than 5 hours, whereas 65% suffer from this at altitude. 11% wake up in their sleep more than 3 times at sea level, and at altitude this figure increases to 54%. Waking up early increases from 11 to 20%. The feeling of not having rested increases from 41% to 61%, and the poor quality of sleep increases from 16% to 61%. At altitude all the ratios improve by the 5th night, but they do not reach the values found at sea level. Thus, the conclusion is that in those cases of intermittent chronic exposure acclimatisation over 4,000m is linked with poor quality of sleep. Its relationship with drowsiness in monotonous work or mental fatigue during next day is unknown.

Table 6
Quality of Sleep, 1st and 5th night at 4300 m & at Sea Level of 122
Acclimatised Mining Workers to HA Intermittent Chronic Exposure.

Mines: Collahuasi, Quebrada Blanca. Shift system: 7x7, 10x10 and 10x7 days.

Age: Mean 38 (20-62) years

Spiegel Test Indexes	Pathologic	
1. Conciliation, falling asleep	: 30 min & (+)	
2. Hours asleep	: 5 hours & (-)	
3. Number of times awakened	: 3 & (+)	
4. Early waking	: involuntary	
5. Awaking perceived	: tired	
6. Quality of sleep	: bad sleep	

Spiegel Indexes	At Sea		At 4,300m	
	1st night	5th night	1st night	5th night
% pathologics				
1. Conciliation	8,3	10,0	45,1	15,9
2. Sleeping Hours	27,8	23,3	65,7	63,5
3. Awakes Number	11,1	26,7	54,9	19,0
4. Early Awaking	11,1	16,7	20,6	11,1
5. Resting Perceived	41,7	26,7	61,4	30,0
6. Sleep Quality	16,7	20,0	61,8	28,6

From Santibáñez *et al*, 1994.¹⁹

A study made by psychologists and ergonomists using specific methodology of the cognitive performance and mental strain on 26 acclimatised workers in ICE, showed that there is a 5% decrease of general cognitive aptitude on the 5th shift day at 4500m

compared with that at sea level¹⁵ (Table 7). The elements most affected are space aptitude and tridimensional performance that decrease by 13%, and mathematical reasoning that drops by 28%. Numerical and verbal aptitude, however, is not significantly affected. In turn, the psychological and psychophysical attention drops by 11% whereas concentration and memory have changes of no statistical significance. Additional studies of cognitive performance during the whole shift and shift change show that for the same performance, more concentration and attention energy is necessary, but with mistakes increasing.

Table 7

Cognitive Performance and Mental Fatigue on 4,500m Intermittent Chronic Exposure Mining Workers. 26 professional and technic acclimatized workers. Mean age: 38 years. Shift System: 7x7; 10x10 days. Normal Personality Test. 7-10th day at sea level and 5th day at 4500 m. Methodology: General Aptitude Test Battery and Digital, DAT and KIM test for attention, concentration and memory.

% Variation at 4500m Compared with Sea Level	
General Cognitive Aptitude	-5
Spacial aptitude	-13
Mathematic reasoning	-19
Numeric aptitude	+3 ns
Verbal aptitude	+6 ns
Mental Test Performance	
Attention	-11
Concentration	+5 ns
Memory	+12 ns

From Rivera, 1993.¹⁵ ns: statistical not significative

Collahuasi has also sponsored studies on the cardiorespiratory response to intermittent chronic exposure; these results are being explained by Dr Jalil *et al* in this symposium.

Special Considerations

It can be concluded that the miners' intermittent chronic exposure is accompanied by physiological responses which are different from those of acute exposure and from those of permanent residence at high altitude. There is evidence that periodic descent to sea level on rest days produce a certain degree of deacclimatization. In fact, during the first 24 hours after arriving at camp to begin a new shift, the workers sleep poorly with lower cognitive performance and with some AMS signs that are partially eased thereafter. But there is still a lot to learn about the evolution of the different parameters of all the work cycle at altitude and the consequences men will experience working under these conditions for ten, fifteen or more years.

The major concern at the moment is to select those who have the best acclimatization response to hypoxia. To attain this we are collaborating in the validation of the

hypoxia test on miners as per the cardio-ventilatory criteria of the Richalet test^{13,14}.

Furthermore, and so as to control the problems that impair the workers' performance, the effect that adding oxygen in bedrooms has on the quality of sleep, cognitive performance and productivity is being studied.

The experience gathered so far enables us to state that the biomedical studies promoted by the Chilean mining industry have contributed to enhancing mountain medicine and the development of multidisciplinary groups for the study of hypobaric hypoxia.

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CHAPTER 26

WORKING AT HIGH ALTITUDE IN ANDEAN MINERS FROM CHILE: HUMAN ADAPTATION TO LONG TERM INTERMITTENT HYPOBARIC HYPOXIA.

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Introduction

Throughout human history, man has lived in different ways at high altitude. Living and adapting to such hypoxic environment classically has two different forms or situations: the first one being the man living permanently at high altitude, as in Perú, Bolivia or in the Himalayas (chronic hypobaric hypoxia). In the second situation are the climbers all over the world which constitutes acute hypobaric hypoxia exposure.

But there is another form of human exposure to hypobaric hypoxia when dealing with human life at high altitude under chronic hypobaric hypoxia. We would like to call this situation the "third situation" which is unique to labor at high altitude. Particularly this happens in high altitude mining, as occurs in the Andes in the north of Chile where several copper, gold and silver mines are located.

In this particular situation, miners work regularly in shifts at high altitude (usually above 3000m and sometimes above 4000m), living and working there for periods as long as 3 to 14 days. Subsequently they descend to lowlands at altitudes regularly lower than 2000 m for 4 - 10 days where they relax and live with their families. These cycles of intermittent hypobaric hypoxia are repeated for a long time (usually for years). In the usual practice, miners arrive directly at the mine from sea level (or sometimes close to it) in 4 - 6 hours by bus which is also different from climbers. Sometimes, but not very often, they spend their first night at an intermediate altitude in order to get a better adaptation.

Most of our knowledge concerning human adaptation to altitudes comes from studies performed under situations 1 or 2 (chronic or acute hypobaric hypoxia). Few data are available in terms of human adaptation to this "third" situation of intermittent hypobaric hypoxia.

We will briefly review some of our data concerning human performance in high altitude mining. These data have been collected during the last four years at two different mining sites in the north of Chile with the purpose of characterizing physiological responses in representative miners under this particular condition.

Methods

Since 1991, demographic, epidemiologic and physiologic data have been collected in miners working at the mines of Choquelimpie (Chile, Arica, Region 1, 4500m) and Collahuasi (Chile, Iquique, Region 1, 4500m). Most of this information has been presented at the Hypoxia 1993 and 1995 Symposia (Lake Louise, Alberta, Canada). Characteristics of both populations are shown on Table 1.

TABLE 1
COMPARATIVE CHARACTERISTICS OF MINERS
AT CHOQUELIMPIE AND COLLAHUASI

Group	Choquelimpie	Collahuasi
n (gender)	93 (men)	64 (men)
Period of Study	1991 - 1992	1993 - 1994
Age (yr)	35 ± 1	35.5 ± 9
Weight (Kg)	75.3 ± 11	72.7 ± 8
BMI (Kg/sqm)	25.5 ± 3.2	25.1 ± 3
Months at work	85 ± 10	55 ± 52 *
Shifts	4*4	10*10 and 7*7
Camp altitude (m)	4500m	4500

mean ± SD

* p < 0.01

In these investigations, substudies were designed to address the following issues:

- prevalence of Acute Mountain Sickness (AMS) by using the Lake Louise Questionnaire 1991¹³.

- modifications in plasma volume and red blood cell number (microhematocrit).
- arterial oxygen saturation at rest and during exercise with an ear oximetry device (Ohmeda II).

- aerobic capacity at sea level and at the work site with progressive treadmill exercise⁷ (Bruce Protocol till exhaustion). A Quinton Q4000 instrument was used.

- non-invasive systolic pulmonary artery pressure measurement with Doppler echocardiography (Hewlett Packard, Sonos 100) of the tricuspid valve, looking for tricuspid regurgitation signal, and calculating systolic pressure gradient and subsequently the systolic pulmonary artery pressure by using Bernoulli's formula.

- vasoconstrictor/antinatriuretic status by measuring plasma renin activity with a radioenzymatic method, plasma aldosterone (radioimmunoassay) and urinary fractional excretion of sodium.

- ventilation at rest and during exercise (with a Quinton, Q-Plex 1 equipment).

Results are shown here as mean \pm SD. For statistical analysis, t-test (for dependent and independent samples), one-way and repeated measurements ANOVA, chi-squared and Fisher tests as well as linear regression analysis were used.

Results

Prevalence of AMS Symptoms. A total of 60 consecutive workers from Collahuasi mine were assessed after their first day and night at the mine^{4,15}. They had been working on that system for at least 6 months before. According to the Lake Louise Scoring System, the mean score value (range from 0 - 15) was 4.7 ± 2 and 53 % of them had moderate AMS (mean score 6.1 ± 1.3) and the other 47 % showed mild AMS (mean Score 2.9 ± 1.1).

Among the 5 main symptoms¹³ of the Lake Louise Questionnaire 1991, symptoms significantly correlated to self-perception of activity impairment were fatigue ($r = 0.64$), gastrointestinal symptoms ($r = 0.43$) and dizziness ($r = 0.46$).

Microhematocrit. In 34 workers from Choquelimpie⁷, microhematocrit at sea level was significantly higher than from a control (age, sex and work) group working only at sea level (Fig. 2) and was not different from days 1 and 4 at 4500m.

Arterial Oxygen Saturation. Resting, submaximal (6 minutes, Bruce protocol) and maximal oxygen saturation were measured by ear oximetry in 34 miners from the Choquelimpie mine working on the 4 by 4 shift⁷. Measurements were performed sequentially at sea level (Arica, Chile), and on days 1 and 4 at the mine.

Arterial oxygen saturation decreased significantly at 4500m at rest and during mild and maximal exercise as is shown on Table 2.

TABLE 2

ARTERIAL OXYGEN SATURATION AT REST AND DURING EXERCISE
AT SEA LEVEL AND AT HIGH ALTITUDE (JALIL 94)

	Sea level	4500 m day 1	4500 m day 4
Resting (%)	97 ± 1	$88 \pm 17^*$	$91 \pm 3^*$
6 minutes (%)	97 ± 1	$85 \pm 6^*$	$86 \pm 4^*a$
Maximal exercise (%)	96 ± 2	$83 \pm 4^*$	$83 \pm 5^*b$

Mean \pm SD

* $p < 0.05$ vs sea level, a = $p < 0.05$ vs resting, b = $p < 0.05$ vs resting and 6 minutes

Aerobic Capacity^{8,2,12}. These workers have very good aerobic capacity at sea level, and at high altitude (4500m). In the study just mentioned^{7,2} total exercise time on a progressive and highly demanding treadmill protocol (Bruce till exhaustion), a significant reduction was observed on days 1 and 4 as compared to sea level. Interestingly, no significant differences were observed between days 1 and 4 (Fig. 3).

Resting and submaximal heart rates were higher at high altitude, but maximal heart rate was significantly lower on days 1 and 4 at 4500m without differences between those 2 days (Table 3).

TABLE 3
HEART RATE AT REST AND DURING EXERCISE AT SEA LEVEL AND AT HIGH ALTITUDE (Jalil 94)

	Sea level	4500 m day 1	4500 m day 4
Resting	64 ± 12	79 ± 12*	73 ± 18*
6 minutes	113 ± 18a	126 ± 18a*	122 ± 18a*
Maximal exercise	179 ± 18b	164 ± 18b	165 ± 18b*

Mean ± SD

* p < 0.05 vs sea level, a = p < 0.05 vs resting, b = p < 0.05 vs resting and 6 minutes

Pulmonary Artery Pressure. As Chamorro et al. have documented, in 35 workers from Choquelimpi, systolic pulmonary artery pressure (PAPs) increased significantly from 19.5 ± 3.5 mm Hg at sea level to 33.4 ± 5.9 (p < 0.01) on day 1 at 4500m^{5,6}. Afterwards, on day 4 at the same altitude, a significant reduction in PAPs, but not to sea level values was observed (Fig. 4). In 22 workers from Collahuasi, PAPs on day 1 was 29.3 ± 9 mm Hg (unpublished observations).

Plasma Renin Activity (PRA). As a way for assessing the circulating renin-angiotensin-aldosterone system in this situation, PRA after 30 minutes of complete supine resting was measured at sea level and on day 1 at 4500m in 8 miners from Choquelimpi¹⁰. A significant reduction in PRA as compared to sea level was observed (from 2.4 ± 0.8 to 0.9 ± 0.3 ng/ml/hr, p < 0.05). In 14 workers from Collahuasi (4500m) a significant increment in PRA was observed from day 1 to day 10, from 1.3 ± 0.3 to 2.6 ± 0.2 ng/ml/hr, p < 0.05¹¹.

Ventilation at Rest and on Exercise at High Altitude. In 23 miners from Collahuasi, Saldías et al have found that ventilatory parameters on day 1 at rest and during maximal treadmill exercise were practically normal, with poor correlation with AMS scores¹⁵.

In conclusion, human adaptation to this "third situation" of chronic intermittent hypobaric hypoxia involves several physiologic changes, at different levels. Most of these changes seem to be intermediate between acute and chronic hypobaric hypoxia exposure. Further information is needed in order to understand these responses. Our current studies are aimed to better characterize these physiologic aspects in this population. There is also a need for improving the risk assessment capabilities⁷ of this population through an epidemiologic surveillance program and to design interventions for improving life quality of these miners.

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CHAPTER 27

LIFE ON THE EDGE: BIODIVERSITY IN CRISIS

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"What in the world is Hackett doing giving a talk on biodiversity?" A good question, deserving an answer. The participants at this conference know me as a high altitude researcher. I am not trained as a conservation biologist, and my limited study of this new field has not rendered me an expert by any means. What compels me to be so bold as to speak on this topic is the importance of biodiversity to all of us, the sense of urgency created by the unprecedented speed at which ecosystems and species are disappearing, and my firm conviction that promoting this issue at the "grass-roots" level is the only solution to the problem. For me, this talk is symbolic, marking a turning point in my career. For more than twenty years, my work (and recreation) has taken me to nearly all the mountain ranges of the world. I have enjoyed the beauty and peace of many wilderness areas, reveled in cultural diversity on six continents, and found sufficient personal and professional challenges to render a degree of satisfaction. On the other hand, I have also seen the results of a fifty percent increase in the world's population over those twenty years—from four to six billion. Environmental degradation has become more and more obvious in my travels, and I don't mean garbage on trekking routes in Nepal. In fact, that sort of polluting has improved over the years, with an increase in the "environmental ethic." It is the overall destruction of ecosystems, the loss of habitat and therefore wild animals, the deforestation and the overgrazing that have become so obvious. My concern propelled me to read enough of the literature on the subject to understand that the mountain ecosystem is only one of the many that are threatened, and although my favorite, it is not the most important ecosystem for world well-being. The scope of the problem is enormous, and the challenge the greatest that we face. But the situation is not hopeless. Progress is being made, and individual efforts can make a difference. Descending from the summit of Mt. Everest, I felt that divine providence had saved me from certain death when I fell, and that perhaps it was for some purpose. I can't think of a better purpose in my life right now than helping to maintain the health of our planet, by spreading awareness of the problem of biodiversity, and urging all of us to action.

* * * *

Introduction

Science does not know how many life forms share the planet with us humans. Estimates range from 10 million all the way to 80 million; only 1.4 million species have actually been identified. Most species live in areas difficult to study, like the

tropical rain forest canopy or the deep ocean. Even in our own yards in North America, however, there are many unidentified species in a single handful of soil.

What is known, however, is that species are disappearing at an alarming rate, a rate comparable to the mass extinctions in the age of the dinosaurs in the late Cretaceous period. Many other species are irreversibly committed to extinction. Edward O. Wilson of Harvard University estimates that 50,000 species are being lost per year in tropical rain forests alone, due to loss of habitat. Other ecosystems with fewer species, such as freshwater lakes and islands, are losing even greater proportions of their life forms. The rate of extinction is several *thousand* times the natural background rate of one to ten species per year, and it is being caused by one species—humans. Entire ecosystems and genetic varieties within species (including both wildlife and domesticated crops) are also disappearing, likely at rates greater than the extinction of species themselves.

Why should disappearing beetles, plants, or birds concern us? To biologists, the question hardly needs asking: a species is the unique and irreplaceable product of millions of years of evolution, a thing of value for scientific study, for its beauty, and for itself. For many people, however, a more compelling reason to conserve biological diversity is likely to be pure self-interest: like every species, ours is intimately dependent on others for its well-being.

Time after time, creatures thought useless or harmful are found to play crucial roles in natural systems. Predators driven to extinction no longer keep populations of potential pests in check; earthworms or termites killed by pesticides no longer aerate soils; mangroves cut for firewood no longer protect coastlines from the erosive force of the sea. Organisms of great use to humankind are continually and often unexpectedly being discovered. Take the example of *thermos aquaticus*, a bacterium that lives in the boiling springs of Yellowstone. This bacterium contains a heat-resistant enzyme that would make medical and scientific history. With the discovery of this new enzyme some thirty years ago, throat bacteria can be cultured and identified within hours rather than days, allowing faster diagnosis and treatment, and saving society a great deal of time, misery, and money—the latter estimated to be worth tens of billions of dollars annually in worker productivity. More recently, the enzyme enabled the final breakthrough in polymerase chain reaction technology, a major advance in molecular biology, again, worth billions of dollars. As conservation biologist Thomas E. Lovejoy, assistant secretary for environmental and external affairs for the Smithsonian Institution, writes of this discovery: "*Thermos aquaticus* is just one example of the importance of biodiversity to human well-being, of how species can and do contribute to both general welfare and economic growth." Dr. Lovejoy's point is that while Yellowstone, the world's first national park, was originally set aside with no notion of preserving biodiversity, it nonetheless serves as a testament to the benefits of such preservation. Diversity is of fundamental importance to all ecosystems and all economies.

In any meaningful strategy to safeguard the world's biological heritage, the top priority is protection of wildlands. Protection of these ecosystems alone will require fundamental changes in the way humanity views and uses the natural world, and a commitment to limit the amount of the earth's bounty that society appropriates for itself. But in order to stave off biological poverty, humanity will have to learn not only to save diversity in remote corners of the world, but to maintain and restore it in the forests and waters that we use, and in the towns and cities where we live.

Few would argue that every beetle, every remaining patch of natural vegetation, or every strain of rice is crucial to planetary welfare. But the dismantling, piece by piece, of global life-support systems carries grave risks. No one has pleaded biodiversity's case better than American wildlife biologist Aldo Leopold did nearly fifty years ago: "If the biota, in the course of eons, has built something we like but do not understand, then who but a fool would discard seemingly useless parts? To keep every cog and wheel is the first precaution of intelligent tinkering."

A more emotional pull toward conserving biodiversity was provided by space exploration. As the great American astronomer Fred Hoyle said a half century ago, "Once a photograph of the earth, taken from the outside, is available, a new idea as powerful as any other in history will be let loose." Now that we have that photograph, what is the compelling idea that has emerged? Significantly, it was a physician, Lewis Thomas, who best answered that question when he wrote in *Lives of a Cell*:

"The most beautiful object I have ever seen in a photograph, in all my life, is the planet earth seen from the distance of the moon, hanging there in space, obviously *alive*. Although it seems at first glance to be made up of innumerable separate species of living things, on closer examination every one of its working parts, including us, is interdependently connected to all the other working parts. It is to put it one way, the only truly closed ecosystem any of us knows about. To put it another way, *it is an organism*."

The thought that our planet is itself an organism—a vast cellular structure living directly from solar energy—is indeed a profound one. Whether one actually believes that the earth is an organism, or a planet that behaves very much like one, one is led to the same conclusion: all the parts are interconnected. The importance of biodiversity, intuited from the photograph of our planet, is that its loss may impact our own survival as a species.

Biodiversity is commonly analyzed at three levels: the variety of ecosystems within which organisms live and evolve, the variety of species, and the genetic variety within those species themselves. **The degradation of whole ecosystems, such as forests, wetlands, and coastal waters, is in itself a major loss of biodiversity and the single most important factor behind the current mass extinction of species.**

Ecosystems.

Tropical rain forest ecosystems, which are believed to shelter at least half the planet's life forms, have been reduced by nearly half their original area. Deforestation annually claims 17 million hectares (one hectare equals 2.5 acres) of the wet and dry tropics, an area about four times the size of Denmark. In Benin, Cote d'Ivoire, western Ecuador, El Salvador, Ghana, Haiti, Nigeria, and Togo, forests have all but disappeared. In most nations, forests have been progressively divided into small fragments surrounded by degraded land, with their ability to sustain viable populations of wildlife and vital ecological processes impaired.

Brazil has more tropical forests and probably more species than any other nation. Massive deforestation continues there, but has slowed appreciably since its 1987 peak, thanks to unusually rainy weather, changes in government policy, and a slowdown in the Brazilian economy. With roughly 90 percent of its groves still standing, by national or international standards the Brazilian Amazon remains relatively intact. In contrast, however, the country's once-vast Atlantic coastal rain forests and coniferous Araucaria forests of the South have been devastated—more than 95 percent destroyed by logging and urban expansion.

Few realize how critical these forests are to biodiversity. Only 1 in 15 to 20 species have been identified, largely because the scientists are in the temperate regions. And when scientists *do* venture into the tropics, the daunting nature of the classification process becomes all too apparent. Consider Harvard entomologist E. O. Wilson's description of the rain forest canopy in Costa Rica:

"Climbing to the top, I could look out over the crowns of all but the highest trees, and peer at the foliage close enough to touch. At my fingertips, literally, as I reached out and pulled a tree branch closer, were squadrons of ants gathered around tree hoppers, thorn-shaped insects busily sucking the juices of tender leaf shoots. The ants were not attacking these strange creatures. They were protecting them from spiders, wasps, and other enemies. In exchange, I knew the tree hoppers deposited sugar-laced excrement for the ants to eat. Such are the bonds of symbiosis that hold the rain forest together."

From a single tree in Amazonia, Dr. Wilson identified 43 ant species, approximately the same number as have been found in all of Great Britain. And when biodegradable sprays are used, typically more than three pounds of specimens comprising as many as 1700 species, mostly ants and beetles, can fall to the ground from the same tree. Similarly, Alwyn Gentry of the Missouri Botanical Garden has identified 283 tree species in only one hectare of forest near Isquito, Peru, compared to a mere 700 species of native tree flora in the *entire* United States. A hectare of Peruvian forest can yield 41,000 species of insects, more than a quarter of them beetles.

Outside the tropics, a number of ecosystem types have been all but eliminated from the planet, including the tall-grass prairies of North America, the great cedar groves of Lebanon, and the old-growth hardwood forests of Europe and North America. Temperate rain forests, less widespread than their tropical counterparts, are probably the more endangered ecosystem. Of the 31 million hectares once found on the earth, 56 percent have been logged or cleared. In the contiguous United States, less than 10 percent of old-growth rain forests survive, scattered in small fragments throughout the Pacific Northwest. In the rain forests of British Columbia, only one of 25 large coastal watersheds has wholly escaped logging.

Wetlands, like forests, are important repositories of biological diversity both near and far from the equator. Among the world's most productive ecosystems, they help regulate water flows, remove sediments and pollutants, and provide essential habitat for waterfowl, fish and numerous other species. They are threatened in many parts of the world by drainage for agriculture or urban expansion, conversion to aquaculture ponds, overgrazing, and in forested wetlands, logging.

Damage to wetlands has been severe in industrial nations, with losses in Italy, New Zealand, and California exceeding 90 percent. Canada contains one-fourth of the world's wetlands, and overall it has lost relatively few. But even here, major losses have occurred: Atlantic salt marshes, prairie wetlands, and Pacific estuarine marshes have been reduced to a third of their original extent. Vast areas of bog and marsh remain in the country's sparsely populated northern regions.

Mangrove swamps, which grow along about a quarter of all tropical coastlines, have suffered heavy losses in Asia, Latin America, and west Africa. In Ecuador, for example, nearly half of these protected wetlands have been cleared, mostly for shrimp ponds, and half of the remainder are targeted for clearing. India, Pakistan, and Thailand have all lost at least three-fourths of their mangroves. Indonesia seems determined to follow suit: in Kalimantan, its largest province, 95 percent of all mangroves are to be cleared for pulpwood production, even though the fisheries nursed by Indo-

nesian mangroves earn roughly seven times as much in export revenue as all their wood and charcoal production combined.

Mangroves and other coastal wetlands form part of an interdependent complex of coastal habitats, protecting those inland from the erosive force of the sea and those offshore from the land-based pollution. Coral reefs, among the most complex and species-rich ecosystems on earth, easily withstand the pounding of ocean waves but are highly sensitive to changes in nutrients, water temperature, and light levels. When fertilizers, sewage, or eroded soil pollute the clear tropical waters where they thrive, these communities of slow-growing animals are often killed off, smothered by sediment or overgrown by fast-spreading algae.

Direct monitoring of **underwater communities** is difficult, but it is likely that coral reefs are in worse condition than either forests or wetlands. The most recent global survey, based on data from the early eighties, found problems such as sedimentation, water pollution, and direct damage from fishers and tourists degrading reefs off 90 of 109 countries. A decade ago, the Phillipine government estimated that 71 percent of that nation's reefs—the most diverse in the world—were in “poor to fair” condition at best; only 6 percent were judged “excellent.” Coastal development and deforestation in the tropics have increased dramatically in the past decade, undoubtedly burdening reefs and other coastal habitats with greater sedimentation and pollution.

Corals are also exhibiting a new kind of degradation: massive bleaching. When subjected to extreme stress, they jettison the colorful algae they live in symbiosis with, exposing the white skeleton of dead coral beneath a single layer of clear living tissue. If the stress persists, the coral dies. Now considered the worst threat to the survival of coral reefs, bleaching occurred without warning at sites throughout the tropics in 1980, 1983, 1987, and 1990. The most serious cases were found in the corals of the Caribbean Sea. While its causes are not known, bleaching appears when ocean temperatures are abnormally high, leading some scientists to call it a harbinger of global warming.

Species

After ecosystems, the second and most familiar category of biodiversity loss is the decline of species. The majority of species (and of extinctions) are invertebrates of the tropical forest, too numerous to identify, let alone monitor. For islands and other habitats with relatively limited numbers of species, the situation is somewhat easier to track. In Hawaii, for example, 41 species of Hawaiian tree snail were listed as endangered by the U.S. government in 1981; today only two remain in substantial numbers, and they are declining rapidly.

Little attention has been paid to conservation of such humble creatures, but their extinction—or even their removal from part of their range—can have profound consequences. Populations of American oyster, which were once so numerous in the Chesapeake Bay that they could filter all the water in the bay every three days, have fallen by 99 percent since 1870. Now, it takes a year for oysters to filter the same amount of water, one reason the bay is increasingly muddied and oxygen-poor.

The Chesapeake bay example makes clear that extinction is only the most extreme form of biodiversity loss. Natural systems, whether on land or in the water, are more than collections of species or genes: they are functioning wholes, processes as well as parts. The American oyster is not considered an endangered species, but its

role in Chesapeake Bay demonstrates that a mere reduction in the local population of the species can disrupt the functions it performs in its ecosystem. The benefits of that system to humanity, such as clean water or seafood harvest, may thus be lost long before extinction becomes a threat.

Fish. In the main rivers and great seas (the Black, Caspian, Aral, and Azov) of the southern republics of the former Soviet Union, more than 90 percent of major commercial fish species have been killed off. Similarly, in peninsular Malaysia, a recent four-year inventory found fewer than 50 percent of the 266 fish species known to have inhabited the region's rivers before the advent of large-scale logging. Introduction of the Nile perch has helped drive half of the 400 species of Lake Victoria, Africa's largest lake, to or near extinction.

Unfortunately for many aquatic life forms, the only measure available to gauge their well-being is how many of them are consumed by humans. This imperfect measure does indicate the widespread over-fishing of many coastal and open-ocean species. Catches of Atlantic cod and herring, Southern African pilchard, Pacific Ocean perch, king crab, and Peruvian anchovies have all declined over the past two decades, according to the UN Environment Programme. Namibian fisheries are on the brink of collapse: in mid-1991 the fisheries department said that a fishing moratorium of at least five years was needed to allow anchovies, mackerel, and other species to recover from "potentially disastrous" levels.

Because most modern fishing techniques are unselective, they often cause greater damage to non-target species than to the ones sought for consumption. Drift nets, the notorious "walls of death" that stretch up to 60 kilometers in length, were recently phased out of use, but other fishing technologies are comparably destructive. For example, the world's shrimp trawls (funnel-shaped nets that are towed behind boats) land nearly 2 million tons of shrimp each year but also snag 2.5 to 10 times that weight in other ocean organisms. Off the northeastern coast of the United States (ironically the most ardent supporter of the drift net ban), boats trawling for yellowtail flounder catch and discard three times as much fish as they keep.

Amphibians. Scientists have discovered an apparent worldwide decline in amphibians in recent years. Because amphibians divide their time between land and water habitats and their skin is permeable to airborne gases, they are especially sensitive indicators of environmental degradation. Habitat conversion and air pollution are likely causes of many species' decline, but amphibians are mysteriously diminishing in seemingly pristine nature preserves. Again, the services provided by nature often become apparent only when they are lost. An adult frog can eat its own weight in insects daily, and diminishing frog populations in India (in part due to European taste for frog legs) have been linked to higher rates of pest damage to crops in Maharashtra State and of malaria in West Bengal. Wetland drainage and invading species have extinguished nearly half of New Zealand's unique frog fauna.

Birds. One indicator of the health of the biosystem is the bird-life. Much like the canaries that coal miners carried into the mines to test for dangerous air, drops in the world's bird species flag environmental dangers that imperil us as well as them. Some 70 percent (about 6,600) of the world's bird species are declining, including 1,000 that are in imminent danger of extinction.

Birds serve not only as beacons of change, however. They are themselves part of the vital life-support system that maintains the health of ecosystems. For example, owls, hawks, and eagles keep small rodent populations in check, while humming-

birds pollinate flowering shrubs, cacti, and trees. Some trees, such as those in the nutmeg family, rely upon hornbills and a few other large fruit-eaters to disperse their seeds through droppings.

Threats to birds are often multiple. Many species are simultaneously jeopardized by habitat destruction and overhunting, trapping, pesticides, oil spills, acid rain, or introduced birds or animals that outcompete or prey on them. A few species—the common pigeon, cattle egret, and common backyard birds, for instance—have prospered in disturbed habitats, giving the impression that birds are abundant. But most species are not. Satellite imagery reveals that expansive habitats are being carved into patchworks, leaving only fragments within which birds must struggle to survive.

Mammals. Almost half of Australia's surviving mammals are threatened with extinction. France, Germany, the Netherlands, and Portugal all report more than 40% of their mammals as threatened. All cetaceans (whales and dolphins) are treated by the Convention on International Trade in Endangered Species as threatened or likely to become so. Virtually all species of wild cats and most bears are declining seriously in numbers. Of the primate species, 116 of the 200 in the world are threatened with extinction.

Plants. Patterns of plant diversity parallel those of animals, with two-thirds of the world's plant species found in the tropics. Although prehistoric extinction spasms tended to claim mostly animals, plants too are now threatened with extinction on a large scale. Peter Raven, director of the Missouri Botanical Garden, has estimated that one-fourth of all tropical plants are likely to be wiped out in the next 30 years.

Outside the tropics, the greatest concentration of threatened plants is found in the arid landscapes of southern Africa. Four-fifths of the plants are endemic (found nowhere else), and 13 percent of these—more than 2,300 species—are reportedly threatened. In the bush of southwestern Australia, another arid region of many endemic species, about two-thirds of the 1,600 plant species are endangered by the rapid spread of a fungal disease carried inadvertently by humans walking or driving throughout the bush. And in the United States, about 3,000 plants, nearly one in every eight native species, are considered in danger of extinction; without strong efforts to save them, more than 700 are likely to disappear in the next 10 years.

Two areas of particular importance regarding plants have to do with food and medicine. Consider the world's food markets. Of temperate zone plants, the fruits of about a dozen species dominate the market. Only relatively recently have tropical fruits like mangoes and papaya joined the northern markets, but there are more than 200 in use in other parts of the world. It is estimated that at least 3,000 may one day be available. These products can be gathered and produced *ad infinitum* without great disturbance of the rain forest, and many can be transplanted to be cultivated in farms elsewhere—just as rubber, cocoa, coffee, brazil nuts, avocado, rattan and many others already have been.

The health problems of three-quarters of the human race are attended to exclusively with medicines derived from plants, while well over half of all pharmaceuticals have a biological, primarily plant origin. On the other hand, fewer than 1% of the world's 250,000 known species of flowering plants have undergone thorough analysis as potential medicines. And while a major barrier to working with plants in the past has been the tedium of manually separating the dozens of chemical compounds in a plant extract—a process that has traditionally taken months—now technological advances enable a laboratory to process hundreds of extracts containing thousands of

compounds in a single week. Even at that rate, however, it is impossible to investigate the species as fast as they are going extinct.

Genetic variety.

Some analysts believe, however, that the greatest threat to human welfare comes from losses at the third genetic level of diversity—the genetic variety within species, most notably food crops and their wild relatives. Farmers have used and created genetic diversity for millennia to increase agricultural production. Crop breeding and genetic engineering (which can only rearrange existing genes, not create them) are no less dependent on it. In developing nations especially, generations of farmers have developed a remarkable array of crops. The Ifugao people of the island of Luzon in the Philippines identify more than 200 varieties of sweet potato by name. Farmers in India have planted perhaps 30,000 different strains of rice over the past 50 years.

The widespread introduction of a handful of high-yielding, or “Green Revolution” crop varieties has boosted overall food production over the past several decades, but eliminated many traditional strains that were well adapted to local ecosystems and could have been used to develop higher-yielding, locally appropriate crops. If current trends continue, three-quarters of India’s rice fields may be sown in only 10 varieties by the year 2005. In Indonesia, 1,500 local varieties of rice have disappeared in the past 15 years, and nearly three-fourths of the rice planted today descends from a single maternal plant. Similarly, in the U.S., just six varieties of corn account for 71 percent of the corn fields, and nine varieties of wheat occupy about 50 percent of the wheat land.

Such high levels of agricultural uniformity leave fields vulnerable to pest and disease outbreaks. At their worst, such outbreaks can rampage over entire countries, as in the Irish potato famine of 1846. In 1991, the genetic similarity of orange trees in Brazil provided the nation’s worst-ever outbreak of infections such as citrus cancer, severely reducing output. Fortunately, some farmers add new crops to their traditional menu without completely abandoning the old. Peasants in the Tulumayo Valley of eastern Peru, for example, raise nearly 180 different varieties of potato, even though nearly half of their fields now grow high-yield potatoes.

Genetic erosion is a problem among wild, as well as cultivated life forms. Through population reductions or intentional homogenization, many species have lost much of their internal diversity, and hence their ability to survive collectively. African cheetahs have almost no detectable genetic diversity, and their uniformity has resulted in reproductive problems and vulnerability to disease. On the west coast of North America, according to the American Fisheries Society (AFS), at least 106 major populations of salmon and steelhead have been wiped out and another 14 types of anadromous fish (those that migrate between fresh water and the ocean) are at some risk. Just as monoculture plantations have largely replaced the region’s forest wilderness, genetically impoverished hatchery fish have supplanted their wild cousins. About 75 percent of the Columbia River basin’s fish are now hatchery-produced and lack the hardiness and survival instincts of wild salmon.

The conclusion of AFS’s Endangered Species Committee could easily apply to the biological heritage of much of the world: “With the loss of so many populations prior to our knowledge . . . the historic richness of the salmon and steelhead resource of the West Coast will never be known. However, it is clear that what has survived is a small proportion of what once existed, and what remains is substantially at risk.”

Global warming.

Some 2.4 million years ago, the earth's climate was cooling and the mammals of eastern Africa faced a challenge. Forests were shrinking and habitats being altered by the changing atmosphere. According to paleontologists' theories, the mammals responded over many millennia by evolving into entirely new species, and whole new families of life were born, including the genus *Homo*, hominids. Today, the only survivor of that lineage, *Homo Sapiens*, has so altered the atmosphere that a global warming, far too rapid for most plant or animal species to adapt, now seems likely.

In the past 150 years, humanity has burned enough fossil fuels and vegetation to increase the amount of heat-trapping carbon dioxide in the atmosphere by 25 percent. The UN-sponsored Intergovernmental Panel on Climate Change (IPCC) predicts that the continuing emissions of carbon dioxide and other greenhouse gases are likely to raise the planet's average temperature roughly 0.3 degrees Celsius per decade, or 1.5 to 4.5 degrees over the next century. Such increases may sound small, but they would take civilization into uncharted territory. A 3 degree warming would raise the earth's temperature to its highest level in 100,000 years; a 4 degree increase would make the earth warmer than it has been for 40 million years.

The magnitude and rate of global warming in store remain uncertain; the impacts of warming on biological diversity are even less predictable. But a safe conclusion from available information is that without major and immediate reductions in greenhouse gas emissions, the impacts of global warming will probably make the world's current biological collapse pale in comparison.

There are recent data indicating that warming may have already begun. After two decades of steadily rising global average temperatures, including the highest on record in 1990, the June 1991 eruption of Mount Pinatubo in the Philippines gave the world a brief respite from global warming. The explosion ejected vast amounts of sulfate aerosols into the upper atmosphere, which quickly spread around the globe. Once there, the aerosols reflected a minute amount of incoming sunlight back into space, enough to exert a cooling effect. By early 1994, however, almost all aerosols had settled out, clearing the way for a resumption of the warming trend. Evidence of new temperature highs was not long in coming. A premonsoon heat wave in central India lasted several weeks with temperatures up to 46 degrees Celsius (115 degrees Fahrenheit), taking a heavy toll on humans and livestock in the region. For the western United States, hundreds of new records were set, creating hot, dry conditions that led to a near record number of forest fires. Japan had the hottest summer on record. Intense heat led to excessive evaporation and water shortages so severe that many utilities and manufacturing firms in Tokyo and surrounding areas were forced to import water by tanker from as far away as Alaska. Over a thousand miles to the west, Shanghai—with little air conditioning—suffered during July through 14 days above 35 degrees Celsius (95 degrees Fahrenheit) and 16 days between 33 and 34 degrees Celsius. And in parts of Northern Europe, including Germany, Poland and the Baltic states, records were set.

A warming world will be hostile to life in countless ways. Most notably, natural communities will be forced to migrate away from the equator, or up from sea level, if they are to maintain their usual climate conditions. For most species and habitats, which have dealt with changing conditions for eons, the warming itself is less a concern than the rate at which it is likely to occur. A century's worth of warming at the IPCC-predicted rate (0.3 degrees per decade, with much greater warming in Arctic

and Antarctic regions) would displace vegetation zones in the world's middle latitudes roughly 300 kilometers away from the equator, or in mountainous regions 500 meters higher in altitude. By comparison, many North American tree species moved 10 to 40 kilometers per century as the last ice age ended 10,000 years ago. The predicted warming, therefore, is likely to result in large-scale diebacks of beech, oak and other deciduous forests. To the north, according to some studies, 40 percent of the world's boreal forests (the woodlands of the far North that cover an area nearly the size of South America) may be killed off by climate changes induced by a doubling of CO₂ concentrations.

Warming will be less near the equator than at higher latitudes, but tropical forests will not be immune to the effects of climate change. Shifting rainfall patterns are expected to disrupt the flowering and reproduction of many tropical plants, thereby affecting many fruit-eating animals. Changes in the timing of rainfall could also make forests more vulnerable to their usual mode of destruction—fires set by farmers and cattle ranchers.

In this process, there may be winners as well as losers. Creatures that reproduce rapidly and thrive in recently disturbed habitats (often called weeds or pests) may expand their ranges at the expense of those that reproduce slowly or require mature or old-growth communities to thrive. One study predicts that sea-grass beds (important fish and waterfowl habitats found along the coastlines of every continent) will expand under CO₂-induced global warming. But oceanic ecosystems as a whole are likely to be greatly disrupted by the combined effects of climate change, even though they will warm more slowly than their on-land counterparts. And since disturbances such as storms, heat waves, and fires may become more frequent and severe with global warming, predictions of biological benefits based on changing average conditions are probably flawed.

The majority of communities and species are likely to suffer from the pace and magnitude of change, and some are especially at risk. Species that inhabit oceanic islands and freshwater habitats on land will generally be unable to migrate across the inhospitable environments that surround their local habitats. They will have to survive climate change in place. Similarly, mountain habitats and those at the poleward edge of continents have nowhere to move. Tundra ecosystems—home to relatively few species but important for many migratory birds—could be reduced throughout North America and Eurasia, releasing CO₂ and the potent greenhouse gas methane as their soil warms. Long-term biological conservation will be impossible without rapid reductions of greenhouse gas emissions, achieved through such measures as energy efficiency and conservation, a shift to renewable energy sources, and the protection and restoration of forests. Unfortunately, these efforts alone will not keep the earth tolerable for its natural and human communities, either. Because of the greenhouse gases already in the atmosphere, a one degree warming, sufficient to cause major biological disruption, appears inevitable during the 21st century.

Ecosystem protection.

For the past century, nature conservation efforts have focused on the protection of habitats in parks and other reserves. This strategy has had an important role in preserving biological diversity. Today there are just under 7,000 nationally protected areas in the world, covering some 651 million hectares, 4.9 percent of the earth's land surface, or 1.3 percent of the earth as a whole. Several nations have, on paper at least,

set impressive proportions of their territory off-limits to development. Bhutan, Botswana, Czechoslovakia, Panama, and Venezuela have designated over 15 percent of their lands as parks. National and global figures, however, mask great unevenness. Parks in Chile, for example, are concentrated high in the scenic Andes, and more than half of that country's unique vegetation types are not protected at all. Globally, high-altitude habitats have received a disproportionate share of protective efforts, while others of greater biological significance (such as lowland forests, wetlands, and most aquatic ecosystems) have been neglected. Belize, for example, protects less than 2 kilometers of its 220 kilometer-long Caribbean barrier reef, the longest in the western hemisphere. The natural systems most commonly found in the world's parks are deserts and tundra.

Although most national parks have been established in the last two decades, indigenous societies have been consciously protecting ecosystems for thousands of years. Although indigenous peoples have proved fully capable of abusing land and hunting wildlife to extinction, it is evident that the world's healthy ecosystems are found predominantly in areas under indigenous control.

Today, both modern and indigenous conservation systems are unraveling. In many areas, such as the Amazon basin, the often intimate knowledge of nature possessed by indigenous people is fading even faster than nature itself. On average, one Amazon tribe has disappeared each year since 1900. Many more have lost their lands or been assimilated into the mainstream culture. Especially for medicinal plants, traditional crops, and other life-forms favored and used by native people, the loss of cultural diversity is one of the greatest threats to biological diversity.

We could never hope to adequately protect biological diversity solely through preservation. The productivity of our land, the diversity of our plant and animal gene pool, and the overall integrity of our forest and stream ecosystems must be protected on commodity-production landscapes as well as in preserves. As plant ecologist Jerry Franklin of the University of Washington writes: "Protection of diversity must be incorporated into everything we do every day on every acre . . ."

An overhaul of humanity's relationship to nature will only be possible with an overhaul of the relationships among people themselves. Because developing nations generally lack the money and the technology to protect their living heritage, and because widespread poverty discourages a fifth of the planet's people from worrying about anything more than immediate needs, financial transfers from North to South are an essential component of conservation. And since much of the devastation of Southern economies and ecosystems is driven by Northern consumption of their resources, then inequities of trade, debt and perhaps most important of all, overconsumption by wealthy nations and individuals, need to be tackled.

In both industrial and developing nations, more funding will be required for nature and genetic conservation. While biodiversity—the most basic of all resources underlying human economies—is a matter of national security, relatively little is spent to secure its future. The \$340 million spent by the U.S. Fish and Wildlife Service to administer the Endangered Species Act over the past 17 years, for example, totaled less than the Sandia National Laboratory spent on nuclear weapons research in 1991 alone. Estimates of the funding needed to implement a global biodiversity convention vary greatly and range as high as \$50 billion annually. But even the most inflated estimates are dwarfed by many of the destructive luxuries the world lavishes money on. World military expenditures in 1991 were \$980 billion.

It is clear that protecting biodiversity will have a cost, as all things of value do; and when the value is incalculable—as in this realm it is—the cost may be steep. It is also clear that there will be immense economic benefits in its protection, and human suffering in its continued loss. The benefits will include money saved when destructive subsidies (for logging, for example) are halted, and when other destructive expenditures (for heavy pesticide use in agriculture, for example) are redirected. The benefits also include the array of existing and new products (medicines, foods, and the like) made available by conserving and studying natural ecosystems rather than blindly exploiting them.

But to try to balance the costs and benefits would be to miss a larger point: life on earth transcends economics, even though economic insights can help choose effective means of halting biological losses. No price can be assigned to the ability of the atmosphere, forests, and oceans together to maintain a life-giving climate; no value can be assigned to a species that has endured for millions of years. Moreover, a viable relationship with the myriad parts and processes of the biosphere lies not so much in any economic sacrifice *for* them as in a recognition of our dependence *on* them. This insight must guide all our activities.

Making the conservation of diversity a goal in everything we do would indeed be a fundamental shift. But anything less will be an abdication of our obligation to pass on to future generations a world of undiminished options, and of our moral responsibility as travelers on the only planet known to support life.

As ethnographer Eugene Anderson notes, “Human beings make sacrifices for what they love.” Those who maintain strong bonds with the biological world on which they depend may be more inclined to make the hard decisions needed to protect it. We, as researchers studying the interaction of humans and other species with the environment, we who depend on the biological world for our livelihood, for our mountain recreation and other activities in “nature”, we are the ones who should be leading the way in the protection of biodiversity.

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ABSTRACTS OF THE NINTH INTERNATIONAL HYPOXIA SYMPOSIUM

1

THE 21-AMINOSTEROID U-74500A PROTECTS AGAINST PEROXYNITRITE INDUCED OXIDATION.

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Nitric Oxide (NO) is an important neurotransmitter in the CNS in physiological conditions. However during hypoxia and reoxygenation NO will react with O₂[•] to form peroxynitrite (ONOO[•]), which has been shown to be very neurotoxic. Peroxynitrite is a powerful oxidant at physiological pH acting like a hydroxyl radical (•OH) and inducing lipid peroxidation which could have major clinical implications. In laboratory and in clinical studies the 21-aminosteroids have been proven to be strong antioxidants as well as good neuroprotective agents. This study examined the effects of a 21-aminosteroid, U-74500A, on the peroxynitrite induced oxidation of deoxyribose and the peroxynitrite induced membrane lipid peroxidation. U-74500A was a good scavenger of the oxidation of deoxyribose (IC₅₀ = 60.9 ± 8.9 μmol/l), it was far more powerful than desferrioxamine (IC₅₀ = 1.1 ± 0.1 mmol/l) and mannitol (IC₅₀ = 3.2 ± 0.2 mmol/l) which have been proven to be effective scavengers of the •OH like activity of the decomposition of ONOO[•]. The peroxynitrite induced membrane lipid peroxidation was effectively inhibited by U-74500A (IC₅₀ = 19.9 ± 3.4 μmol/l), again being more active than desferrioxamine (IC₅₀ = 84 ± 3 μmol/l). Mannitol could not inhibit the membrane lipid peroxidation. Our data show that U-74500A is a powerful scavenger of peroxynitrite induced oxidative reactions. 21-Aminosteroids like U-74500A now become available for clinical use and can be regarded as very promising drugs in the prevention and therapy of neuronal damage caused by hypoxia and ischemia.

2

HYPOXIC PULMONARY VASOCONSTRICITION IS NOT NECESSARY FOR DEVELOPMENT OF RADIOGRAPHIC EVIDENCE OF PULMONARY EDEMA FOLLOWING EXERCISE AT MODERATE ALTITUDE.

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High altitude pulmonary edema (HAPE) following rapid ascent to altitude is often associated with heavy exertion. Hypoxic pulmonary vasoconstriction (HPV) is thought to play an important role in HAPE. The purpose of the present study was to determine whether HPV is necessary for the development of pulmonary edema. Thirteen healthy, well trained cyclists were taken to ~2440 m where they performed either 90 or 132 km strenuous exercise. Chest roentgenograms (CXR) were performed before and immediately following exercise. Radiographs were coded and analyzed for signs of pulmonary edema (e.g. peribronchial cuffing). Resting echocardiograms were obtained in 5 of these cyclists while breathing room air and 10-20 min breathing 12-13% oxygen in nitrogen. Estimated pulmonary artery pressure was determined from the echocardiograms by an echocardiographer without knowledge of the chest radiograph results. All subjects had normal baseline LV function and ejection fraction. Tricuspid regurgitation (TR) velocity increased during hypoxia in 3 subjects, remained the same in 1 and decreased in 1. Four of the 5 subjects for whom echo results were available demonstrated peribronchial cuffing. The subject with no radiographic changes showed no increase in TR velocity, but the subject with a decrease in TR velocity did have CXR evidence of edema. HPV may be important in the full HAPE syndrome, but these preliminary data suggest that CXR evidence of early pulmonary edema is not dependent on an increase in pulmonary artery pressure.

3

INTRACRANIAL FLOW VELOCITIES (IFV):
RESPONSES TO VALSALVA'S MANEUVER(VM);
EFFECTS OF AGE ALTITUDE AND ETHNICITY.

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Instituto C. Mondino, Pavia, Italy.

Subjects(S's) studied at 1500m(23), 2200m(12), 3100m(6), 4100(19), 4700m(12), 5100m(17) ages 19-68, (Natives, N's 22-30). Chronic studies after 13 days(17) at 3700m; after 6 days(6) at 3100m. End tidal pCO_2 before, during and after VM. Middle cerebral(MCA) and vertebral(VA) arteries IFV by TCD. Valsalva ratios VR's (MCAVR, VAVR)=highest IFV post/lowest IFV at end of VM adjusted for pCO_2 . MCAVR and VAVR inversely related to altitude. MCAVR, VAVR< with chronic exposure. VAVR>MCAVR. VR's not related to age. VR's adjusted for chronicity, pCO_2 40 Torr and pre-VM pCO_2 equalizes MCAVR and VAVR. N's < VR's than S's. No difference in VR's between trained and untrained S's. VR's independent of pCO_2 ; dependent on change in intracranial pressure(ICP) induced by VM. Altitude headache primarily ICP dependent.

Supported by grants from NMHEMC Research Foundation and Inst. C. Mondino.

4

FREQUENCY DOMAIN ANALYSIS OF HEART RATE VARIABILITY (FDHRV): EFFECTS OF ALTITUDE AND PROLONGED EXERTION.

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We analyzed FDHRV in Tarahumara Indians(TR) and American ultra runners(AC) at moderate altitude before and after a 161km trail race. TR's(n=5) mean age 23.2(range 19-29), AC(n=11) mean age 46.3(range 33-63). TR's<AC's($P=0.001$). Data acquired for 10 min. (Colin® Tonometer), average altitude 3100m reaching 3800m. FDHRV before race, ratios high/low frequencies, TR's=1.36±0.2; AC's=3.44±0.7 ($P=0.03$). Age was inversely related to ratios in AC's. Post-race ratios were not significantly different in TR's and AC's, both groups aged, TR's>AC's ($P=0.05$). American ultra runners (like untrained subjects) have an age related decrease in high frequency spectral peaks. Ethnic differences in spectral peaks are present at rest. Prolonged exertion at altitude causes "aging" which is more pronounced in younger athletes.

Supported by grants from NMHEMC Research Foundation and Inst. C. Mondino.

5

POSSIBLE ROLE OF INCREASED VENTILATION IN SYMPATHETIC ACTIVATION AT HIGH ALTITUDE

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Sympathetic nervous activity, as suggested by elevated plasma norepinephrine (NE), increases over time during high-altitude sojourn, yet the mechanisms of this response remains unknown. To examine the relation between sympathetic activation and ventilatory adaptation, we performed serial measurements of 24 hr urinary catecholamine excretion and resting ventilation in 11 healthy male volunteers (26±1.2 yr) at sea level and over a 21 day sojourn at 4,300 m. Six of 11 subjects were given propranolol (240 mg/day). Epinephrine excretion was not altered at 4,300 m. NE excretion increased over time as minute ventilation increased with resultant decrease in PETCO_2 and increase in PETO_2 at 4,300 m. A positive correlation of NE excretion to PETO_2 existed at 4,300 m, but was eradicated when sea-level data were included. However, NE excretion correlated positively to minute ventilation ($r=0.67$, $n=20$, $p<0.01$) and inversely to PETCO_2 ($r=-0.90$, $n=20$, $p<0.001$) for all measurements including sea-level data. Propranolol treatment did not affect any correlations. Thus sympathetic activation at 4,300 m was not directly related to the hypoxic stimulus, but was related to ventilatory parameters. Increased ventilation may be a stimulus for enhanced sympathetic activity over time during high-altitude sojourn.

6

PLASMA VOLUME CHANGES AFTER ACTIVE BUT NOT PASSIVE ASCENT TO HIGH ALTITUDE

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Inconsistent changes in plasma volume (PV), possibly related to the degree of acute mountain sickness (AMS), have been reported after acute exposure to high altitude. Therefore, we examined the role of both hypoxia and exercise on PV at high altitude. We measured the PV at 550 m and 4 to 5 weeks later at 4559 m, 18 to 20 h after passiv (by helicopter, AIR-group, $n = 8$) or aktiv (FOOT-group, $n = 9$) ascent in healthy mountaineers. Since fluid shifts between the intra- and extravascular compartment may be caused by changes in the capillary permeability, we also determined the transcapillary escape rate of albumin (TER). PV (ml/kg) and TER (%/h) were calculated from the linear decline of the plasma radioactivity after i.v. injecting 5 μ Ci [125I]-albumin (Ballmer P.E. et al. Metabolism 1994; 43: 697). In the AIR-group, PV was 43.6 ± 6.9 (mean ± sd) at low compared to 42.1 ± 4.8 ml/kg at high altitude ($p = 0.4$). In contrast, PV increased significantly in the FOOT-group from 43.8 ± 7 to 48.3 ± 6.6 ml/kg after ascent ($p < 0.01$, by Wilcoxon signed rank test). Total plasma protein concentrations (AIR-group: 70 ± 4 vs 72 ± 3 g/l; FOOT-group: 70 ± 3 vs 70 ± 3 g/l), colloid oncotic pressure (AIR-group: 25.8 ± 1.8 vs 26.3 ± 1.4 mmHg; FOOT-group: 25.6 ± 1.8 vs 26.5 ± 1.6 mmHg) and TER (4.9 ± 1.8 vs 6.3 ± 1.7 %/h in the AIR-group and 4.7 ± 1.1 vs 6.1 ± 1.5 %/h in the FOOT-group; $p = 0.1$) showed no significant changes in both groups. AMS scores were not significantly different between the groups and did not correlate with changes in PV and TER. The pooled data from our previous study ($n = 41$) showed also no change in TER between low and high altitude (6.1 ± 2.1 vs 6.3 ± 1.8 %/h; $p = 0.5$). In conclusion, independent of the presence or absence of AMS, high altitude exposure does not cause a systemic increase in capillary permeability as shown by the unchanged TER. The increase in PV in the FOOT-group strongly supports that exercise and not hypobaric hypoxia or AMS are responsible for the iso-oncotic expansion of the intravascular space.

ABSTRACTS

7

SEX DIFFERENCES IN BLOOD GASES DURING ACCLIMATISATION

Barry PB, Mason NM & Collier DJ, British Mount Everest Medical Expedition c/o DJC, Clinical Pharmacology, St Bartholomew's Hospital, London, EC1A 7BE.

There have been few large-scale studies of blood gas measurements during acclimatisation. This study investigated the changes in capillary blood gas tensions on arrival at 5300m and after acclimatisation to this altitude. We studied 29 members of the BMEME on arrival at 5300m, and 33 members after acclimatisation to this altitude, using a Ciba-Comin 248 blood gas analyser. During acclimatisation pCO_2 fell from an average of 3.46kPa to 3.27kPa ($p<0.05$), the decline was greater in the women studied to 2.85kPa (n=6) c.f. 3.34kPa in the men (n=28) ($p=0.025$).

Capillary oxygenation improved with time at 5300m, from a mean of 5.83kPa on arrival to 6.00kPa after acclimatisation ($p=0.06$), but although women showed a greater improvement, to a mean of 6.18kPa c.f. 5.97kPa in the men, this effect did not approach significance ($p=0.4$). Capillary pH did not alter overall, from a mean of 7.43pH units to 7.44pH units after acclimatisation. Bicarbonate estimations showed little overall fall from a mean of 19.8mmol to 19.5mmol after acclimatisation (n.s.), but as you would predict from the above, bicarbonate fell significantly more in the women studied, to 18.6mmol c.f. 19.6mmol in the men ($p<0.05$). The same was true of estimates of base excess, which showed a greater decline in the women, -7.0mmol c.f. -5.6mmol in men ($p<0.02$). As both men and women appeared to have maintained acid-base homeostasis well, the results suggest that ventilatory control changes in chronic hypoxia may be superior in women.

8

MENSTRUAL CYCLE PHASE DOES NOT AFFECT WORK PERFORMANCE AT SEA LEVEL AND 4,300 M

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We hypothesized that progesterone mediated ventilatory stimulation during the mid-luteal phase would increase arterial O_2 saturation (SaO_2 %), enhance O_2 transport, and increase submaximal exercise time to exhaustion (EXH; min) at 4,300 m. Seven female lowlanders ($X \pm SD$; 34 ± 4 yr, 57 ± 7 kg, 26 ± 4 % fat) exercised to exhaustion at 70% of altitude-specific peak O_2 uptake ($VO_{2\text{peak}}$; $\text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) at sea level (SL) and with acute exposure to 4,300 m simulated altitude (AA) during both the follicular (F) and mid-luteal (L) menstrual cycle phase. Rated perceived exertion (RPE) and SaO_2 were taken after 45 min exercise.

Condition	$VO_{2\text{peak}}$	EXH	SaO_2	RPE
SLF	46.8 \pm 4.5	100.3 \pm 48.9	96.6 \pm 1.1	15.0 \pm 1.5
SLL	46.2 \pm 6.1	112.8 \pm 53.4	96.0 \pm 1.1	13.6 \pm 3.3
AAF	33.4 \pm 3.9*	107.2 \pm 49.0	72.4 \pm 2.6*	13.9 \pm 3.3
AAL	33.8 \pm 3.0*	100.1 \pm 34.2	75.4 \pm 4.1**	14.6 \pm 2.3

* $p<0.05$ from SL. ** $p<0.05$ from F. SaO_2 was significantly increased (3%) in the mid-luteal compared to the follicular phase of the menstrual cycle at AA. This increase in SaO_2 did not increase EXH. We conclude that submaximal and maximal work performance are not affected by menstrual cycle phase at SL or 4,300 m.

9

BAROREFLEX CARDIOVASCULAR MODULATION ; POWER SPECTRAL ANALYSIS(PSA); ALTITUDE, BREATHING AND ETHNICITY.

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Sojourners(10), Natives high-low altitude (3), Low altitude yogis(6), studied at 200m, and 4970m, acutely and 5 days later. High altitude native villagers(10) at 4660m. Blood pressure(BP) by Colin® tonometry, EKG, controlled (yoga) respiration(YR), neck suction(NS) (carotid baroreceptor, [CB's], stimulation,), photoplethysmography of finger (PPM). Signals digitized, on-line analyzes by auto regressive PSA. Altitude, reduction in sympathetic low frequency and (mechanical) high frequency components in PPM, variances of R-R decreased, increase in BP but no change in variance. YR increased BP variability at 200m and 4970m.. NS similar to YR. BR's active at altitude, counteract sympathetic activation, YR and NS similar effects on PSA. Supported by: NMHEMC Research Foundation and Instituto C. Mondino.

10

IN VIVO 2 D 1H COSY NMR STUDY OF METABOLIC RESPONSES TO ACUTE CEREBRAL HYPOXIA

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We have directly monitored, *in situ*, by the 2 D 1H correlation spectroscopy (COSY) technique, the changes in brain metabolites that occur during hypoxia and recovery. Male Wistar rats (300 g, n=5) were studied during 50 min hypoxia at 35 mmHg PaO_2 . COSY spectra were obtained using a SUPER-COSY sequence ($TE = 86$ ms, $NS = 8$). After 50 min of hypoxia, the glucose signal decreased dramatically, as metabolism shifted to lactate production. The Aspartate signal was much smaller (83 ± 3 %) and the Glutamate-Glutamine signal remained significantly lower (30 ± 5 % than control). The arterial pH moderately decreased (7.26 ± 0.04) and the MABP and ECG fell significantly (50 ± 5 %). These metabolic and systemic perturbations were quickly reversed once a normal oxygen supply was restored. The rise in tissue lactate content and the drop in intracerebral glucose may be due to increased glycolysis induced by hypoxia. The fall in Asp may be explained by a shift in the aspartate aminotransferase reaction being triggered by a redox-dependant rise in the production of TCA cycle intermediates. The decrease in Glu-Gln may have resulted from decreased production linked to the anaerobic consumption of glucose, plus the extracellular release of Glu where it may be lost by decarboxylation to GABA or by amidation to glutamine.

11

EFFECTS OF DIETARY PROTEIN LEVEL ON ALTITUDE-INDUCED MUSCLE WASTING IN RATS

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Muscle growth, muscle and liver glycogen, and plasma hormones were evaluated in 11rats chronically exposed during 26 days to hypobaric conditions (HA; $P_b = 354\text{ mmHg}$, 6,000 m), 2) maintained under normobaric conditions, either fed *ad libitum* (SL) or 3) pair-fed equivalent quantities of food consumed by HA animals (PF). All animals were fed diets of varying protein concentrations (10%, LP; 20%, MP; 40% protein, HP). Body weights of HA animals were significantly lower than those of PF rats, consuming either LP, MP, or HP diets (-6%, -8%, -9%, $P < 0.01$, respectively). A specific effect of hypoxia on muscle atrophy has been identified by comparison of muscle weight-to-body weight values between HA and PF groups ($P < 0.05$ for all dietary protein levels). Serum insulin concentrations were lower in HA than in SL and PF rats ($P < 0.05$). Liver glycogen was significantly decreased by both exposure to HA ($P < 0.001$) and high dietary protein content ($P < 0.005$). Hypoxia *per se* and decreased food intake have additive effects on soleus muscle glycogen concentrations. Increasing dietary protein content had deleterious effects on glycogen deposition in EDL muscle ($P < 0.001$). These results clearly demonstrate that increasing the dietary protein intakes in rat, did not minimize the muscle wasting related to HA exposure, but had deleterious effects on glycogen deposition in tissue.

12

 β -ADRENOCEPTORS CONTRIBUTE TO HYPOXIA-MEDIATED VASODILATION IN MAN

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In this study the effects of hypoxia (SpO_2 , 80%) on forearm blood flow (FBF), plasma (nor-)epinephrine levels, and β -adrenoceptors were investigated in 7 healthy volunteers. The experiments were performed in a ventilated hypobaric chamber. The brachial artery of the nondominant arm was canulated for local intra-arterial infusion of propranolol 100 ng/kg/min, blood pressure recording, and blood sampling. An antecubital vein of the contra-lateral arm (control-arm) was canulated for venous sampling. FBF was measured on both arms by venous occlusion plethysmography before and during hypoxia. Forearm vascular resistance (FVR) was calculated. During normoxia propranolol did not influence FVR. Hypoxia induced a decrease in FVR of $-24 \pm 6\%$ ($p < 0.05$) in the control-arm, whereas in the infusion-arm (propranolol) a significant increase in FVR was observed ($38 \pm 11\%$; $p < 0.01$). Both arterial and venous (nor-) epinephrine levels were not significantly influenced by hypoxia. These results show that during hypoxia β -receptors are activated, mediating vasodilation. Since arterial and venous (nor-)epinephrine levels were unaltered this indicates that hypoxia increases the sensitivity or the number of the vascular β -adrenoceptors.

13

GENERALISED PROTEIN LEAKAGE IN ACUTE MOUNTAIN SICKNESS

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AMS associated albuminuria is caused by increased glomerular permeability together with decreased tubular reabsorption of albumin. The underlying mechanism could be due to local renal factors or part of a generalised phenomenon also causing cerebral and pulmonary oedema. To assess this further, eight subjects were investigated before and after a helicopter ascent from 1,500M to 4,600M. Urine and salivary albumin and IgG concentrations were measured daily for five days together with AMS symptoms questionnaires. Albumin and IgG concentrations increased several fold in all urine samples for 24-48 hours after ascent. Albuminuria occurred before IgG loss (Mol. Wt. 65 Kd and 150 Kd respectively) suggesting that capillary pores continued to increase in size over 24 hours. Similar changes occurred in saliva albumin/IgG ratios. There was a correlation between increased protein leakage, clinical symptoms and leg capillary filtration coefficient measured in a parallel study. In two control subjects on acetazolamide protein leakage and symptoms were minimal. These findings suggest that there is a generalised capillary leakage in AMS.

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PROGESTERONE IN ACUTE MOUNTAIN SICKNESS

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Comparison was made of daily acetazolamide (Az) 500mg, progesterone (Pg) 60mg, Az 500mg plus Pg 60mg (Az+Pg) and placebo (Pl) in 24 subjects trekking to 5,200m over 7 days for the prevention of acute mountain sickness (AMS). Medication was commenced 7 days before ascent. Assessment comprised clinical interview twice daily, AMS self questionnaires (BMRES and Lake Louise); PaO_2 , PaCO_2 and pH from arterised ear lobe samples and an exercise test. Progesterone subjects had less symptoms of AMS than other groups ($P < 0.005$) and Pl more symptoms ($p < 0.005$) whilst PaO_2 was higher in the Az+Pg group ($p < 0.02$). The exercise test showed greater performance in the Az and Pg subjects ($p < 0.05$) but Az+Pg were similar in performance to Pl. Side effects were graded Az+Pg>Pg>Pl with paraesthesiae the most frequent symptom. In conclusion, Pg can prevent AMS and appears similar to Az in effectiveness.

Supported by The Wellcome Trust, Arthur Thompson Trust and Ciba Corning Diagnostics UK Ltd.

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PULMONARY ARTERY PRESSURE MODULATION AS AN ALTITUDE SIMULATION TOOL: RESPONSES IN HEALTHY PEOPLE

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Simulation of altitude conditions constitutes a way of testing physiologic responses to this hypoxic environment. In this study the response of the systolic pulmonary artery pressure (PAPs, mm Hg) to low oxygen has been measured in a non-invasive way in order to establish its sensitivity to changes in arterial oxygen saturation in normal people. Pulmonary artery systolic pressure was measured by continuous wave pulsed Doppler echocardiogram of the tricuspid valve in 10 normal male volunteers (mean age: 30.1 ± 5.8 years), before and after (12 ± 7 minutes) the administration of a low oxygen concentration (8.12) through a Rudolph mouthpiece. The study was performed in Santiago (740 m) and arterial oxygen saturation (Ox sat, %) was monitored through ear oxymetry. Results were (mean ± SD):

	PAPs	Ox sat
Baseline	22.2 ± 2.7	97.8 ± 1.2
At maximal Ox	33.8 ± 5.8*	77.2 ± 9.1*

*p < 0.01 vs Baseline

A negative correlation was observed between PAPs and Ox sat ($r = -0.78$, $p < 0.01$, $n = 50$). These results reproduce previous observations in normal people at altitudes close to 4500 m and suggest that this tool may constitute a useful way of testing their physiologic degree of adaptation to such hypoxic conditions.

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HYPOTONIC OR HYPERCAPNIC RESPONSE?

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In a series of studies of hypoxia examining the role of adenosine antagonists (Am Rev Respir Dis 1991; 143:A655) and hormonal responses to acute prolonged hypoxia (Am Rev Respir Dis 1991; 143:A785), we noted that there was no significant ventilatory response in 12 healthy male subjects in the supine position in response to breathing 11% O₂ (balance N₂) over a 30 minute period. There was an initial, marked fall in arterial O₂ saturation (SaO₂), and a slower continued decrease in SaO₂ over the next 30 minutes. The SaO₂ at various time points varied widely between subjects. However, there was no significant or sustained increase in minute ventilation (V_E) or in the end-tidal CO₂ concentration (PetCO₂).

Baseline	Immed	5 min	10 min	30 min
SaO ₂ 96.5 ± 1.7	91.8 ± 2.3	82.8 ± 3.8	80.1 ± 5.1	75.1 ± 6.5
V _E (l/min) 8.5 ± 2.5	9.6 ± 2.9	9.7 ± 2.6	9.4 ± 2.7	9.7 ± 1.7
V _T (l) 0.72 ± 0.24	0.75 ± 0.24	0.75 ± 0.24	0.68 ± 0.22	0.66 ± 0.21
PetCO ₂ 40.3 ± 2.1	39.3 ± 2.0	38.5 ± 2.3	37.7 ± 2.8	38.3 ± 1.9
mmHg				

When the same experiment was repeated with an attempt to maintain the PetCO₂ at 40 mmHg, there was a significant increase in V_E in many of these subjects. These results would imply that in man, there is perhaps no significant ventilatory response to induced poikilocapnic hypoxia, and the ventilatory responses previously noted may be an expression of increased sensitivity of the CO₂ ventilatory response in the presence of hypoxia.

ABSTRACTS

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ADAPTATIONS TO HYPOXIC, ACID AND HYDROGEN SULPHIDE RICH WATER IN THE ARMoured CATFISH, *Hoplosternum littorale*.

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H. littorale is a facultative air-breathing catfish from the Amazon, which is tolerant to hypoxic, acidic and hydrogen sulphide (HS) rich waters. Air breathing in fishes is known to be an important strategy to surviving hypoxia but it's importance to surviving in acid and HS rich waters has not previously been investigated. Air breathing frequency (ABF) in *H. littorale* increased from 2 to 28 breaths⁻¹ (B⁻¹) as water PO₂ was reduced from 155 to 100 mmHg. Further reductions in PO₂ elicited a decrease in both ABF and metabolic rate. During acid water exposure (pH=2.8, PO₂=155 mmHg), ABF increased to 28 B⁻¹ and during exposure to 700 μ M HS (buffered to pH 5.6, PO₂=155 mmHg) ABF increased to 40 B⁻¹. In fish denied access to air 200 μ M HS is lethal. Thus, air breathing in the armoured catfish may be important to survival in HS and acid waters while reductions in metabolic rate are preferred during exposure to hypoxia. "Friagem" is a local, annual event in the Amazon, where hypoxia, acid and HS waters occur simultaneously. *H. littorale* is one of the only species which can tolerate friagem, possibly due to a combination between air breathing ability and reduction in metabolic rate.

This research was supported by IDRC of Canada.

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HEART RATE VARIABILITY DURING SIMULATED DESCENT FROM HIGH ALTITUDE WITH PORTABLE HYPERBARIC CHAMBER.

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To evaluate if correction of high altitude hypoxia may influence neurosympathetic balance we studied three subjects during the Himalayan Expedition "Guide del Monteros 93" to Churen Himal (7,371 m). The day of arriving to Base Camp (4,150 m) (Basal) and after 48 hours (Acclimatization), the subjects underwent continuous Ecg Holter monitoring. Two consecutive phases were marked: clinostatic rest (Ph1) and simulated descent with portable hyperbaric chamber down to 2,200 m (Ph2). O₂ Saturation (SatO₂%) has been also recorded with continuous pulseoximeter device (Pulseox 3 Minolta). In each phase, 300 consecutive normal beats with stable signal were analyzed in time domain (mean RR, RRMSD) and frequency domain (Total variance, High Frequency, Low Frequency) by means of auto regressive spectral analysis (Remco Italia Cardioline). Analysis of variance for repeated measures showed no interactions between Basal-Acclimatization x Ph1-Ph2, no difference between Basal vs. Acclimatization. The differences between Ph1 vs. Ph2 are shown in the following table:

	Phase 1	Phase 2	p < .		
Mean	±	SD.			
RR mean (msec)	863	± 174.57	916	± 152.28	0.02
SD RR m	53.83	± 24.01	68.11	± 26.66	0.02
Var/Tot(msec ² Hz ⁻¹)	3487	± 2724	6274	± 7501	
HF NU	17.32	± 10.34	45.69	± 21.33	0.005
LF NU	69.33	± 16.18	48.35	± 23.55	0.03
LF/HF	5.7	± 3.71	2.23	± 3.73	0.02
Sat O2%	88.5	± 1.72	97	± 1.41	0.01

*Analyzed after logarithmic transformation

In conclusion: simulated descent with portable hyperbaric chamber from 4,150 down to 2,200 m has determined a significant improvement of SatO₂% and a concomitant redistribution of sympathetic and parasympathetic indices.

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VENTILATORY PERFORMANCE AFTER CLIMBING AT HIGH ALTITUDE

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To evaluate the ventilatory pattern during the Himalayan expedition "Guide del Monterosa '93" to Charcha Hinul (7,371 m), forced expiratory volume in one second (FEV1), forced vital capacity (FVC), FEV1/FVC ratio, peak of the expiratory flow (PEF), tidal volume (TV), minute ventilation (MV) and transcutaneous oxygen saturation (SatO2%) were recorded in 4 male climbers. Portable spirometer (Pocket II, Micromedical Inc.) and continuous pulseoximeter device (Pulsiox 5, Mindota) were used. Measurements were taken at rest at Base Camp (4,150 m) before and after two different weeks (a and b) of climbing at higher altitudes. Data were collected the first day (B), 16 (16a) and 40 (40a) hours after the first week of climbing at different altitudes (maximum 5,500 m), and 1 (1b) and 16 hours (16b) after the second week of climbing (maximum 6,600 m). Results are shown in the following table.

	B	16a	40a	1b	16b
FEV1	4.4±0.8	3.1±1.1 **	4.3±0.7	4.1±1.0	2.1±0.8 *
FVC	5.5±0.9	4.6±1.0 **	5.5±0.7	5.1±1.0	3.0±1.2 *
PEF (l/s)	10.3±1.3	7.4±2.8 **	10.5±1.3	9.9±2.0	5.1±2.0 *
FEV1/FVC	0.80±0.05	0.74±0.08	0.77±0.04	0.80±0.05	0.73±0.08
TV	1.0±0.2	0.9±0.2	1.0±0.1	0.8±0.1	1.1±0.2
MV (l/min)	12.2±3	13.3±3	14.1±1	14.2±2	16.2±2
RR	12±1	13±2	15±1	16±1	15±5
SatO2%	89±1	88±2	89±1	88±1	89±2

*p<0.001 versus Basal, 1a, 40a, 1b. **p<0.05 versus Basal, 1a, 40a, 1b.

The significant decrease in respiratory performance at 16a and 16b indicates that a predominant restrictive pattern occurred. However, this functional alteration did not appear immediately after the effort. This event could be determined by a possible lung interstitial transient oedema probably caused by water retention following rehydration after prolonged effort at high altitude.

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ACUTE MOUNTAIN SICKNESS IN MINERS EXPOSED CHRONICALLY TO INTERMITTENT HYPOBARIC HYPOXIA

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Symptoms of acute mountain sickness (AMS) may be disabling in terms of physical activity and specially at work. In order to characterize prevalence of AMS and its profiles in altitude miners we have quantified AMS symptoms on the first day at 4500 m in 60 adapted Chilean male workers (mean age: 35.4 ± 8.8 years) on periodical cycles with shifts at 4500 m (Collahuasi, Chile) followed by resting periods under 3000 m. The Lake Louise Consensus 1991 questionnaire was applied. Results were:

scale mean SD

fatigue	(0-3)	1.2	0.7
headache	(0-3)	0.67	0.63
GI symptoms	(0-3)	1	0.66
sleeping abnormalities	(0-3)	1.22	0.98
dizziness	(0-3)	0.63	0.66
total score	(0-15)	4.68	2

53 % of the workers showed moderate AMS (total score > 4, mean: 6.1 = 1.3). Significant ($p < 0.01$) correlation between total score and fatigue ($r = 0.49$), sleeping abnormalities ($r = 0.56$), dizziness ($r = 0.52$) and GI symptoms ($r = 0.60$) was observed. Perception of activity impairment was correlated with fatigue, dizziness and GI symptoms ($r = 0.64$, 0.46 and 0.43 respectively, $p < 0.01$). Thus, moderate AMS is rather common in altitude mine workers even if they are considered as adapted. These symptoms are related to activity impairment and probably to productivity.

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EFFECTS OF HYPOCAPNIC/ISOCAPONIC HYPOXEMIA AND HYPERVENTILATION ON RENAL FUNCTION IN NORMAL, WATER-LOADED VOLUNTEERS.

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Departments of Anesthesia and Clinical Physiology, Herlev Hospital, University of Copenhagen, DK-2730 Herlev, Denmark. This study tested the hypothesis that acute hypoxic hypoxemia increases proximal tubular outflow and sodium clearance (C_{Na}) to the same extent as hypoxic normoxemia, and that this response is blunted during isocapnic hypoxemia. Eight subjects were investigated on five occasions: Inhalation of 10% O_2 , hyperventilation of room air to CO_2 values similar to hypoxic hypoxemia, inhalation of 10% O_2 with CO_2 supplement to produce isocapnia, and, finally, room air with and without the use of the face mask. Lithium clearance (C_L) was used as an index of proximal tubular outflow. Compared with isocapnic normoxia, ERPF increased during hypoxic and isocapnic hypoxemia and hyperventilation, but changes in GFR were not significant. FE_L increased and proximal tubular reabsorption decreased during hypoxic hypoxemia and hyperventilation, but were unchanged during isocapnic hypoxemia. However, compared with baseline, C_{Na} increased during hypoxic and isocapnic hypoxemia, hyperventilation, and isocapnic normoxemia with, but not without the face mask. In conclusion, acute hypoxic hypoxemia decreases proximal reabsorption secondary to hyperventilation and decreased CO_2 tensions. However, application of a face mask may by itself by other mechanisms increase C_{Na} .

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HYPOXIC PRESSOR RESPONSE IS DEPENDENT ON NEURONS IN THE C1 AREA OF THE ROSTRAL VENTROLATERAL MEDULLA

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Neurons of the C1 area of the rostral ventrolateral medulla project to the intermedialateral cell column of the thoracolumbar spinal cord and contribute to the maintenance of vasoconstrictor tone by providing tonic excitation to sympathetic preganglionic neurons. Previous studies have demonstrated that C1 area neurons are activated by stimulation of arterial chemoreceptors. The hypothesis of this study was that neurons in the C1 area are essential for the expression of the pressor response to stimulation of arterial chemoreceptors. Experiments were performed on eight mongrel dogs anesthetized with alpha-chloralose/urethane and mechanically ventilated with room air. Selective stimulation of arterial chemoreceptors was accomplished by ventilation with 100% N_2 for 6 breaths and by intravenous infusion of sodium cyanide (NaCN, 50 μ g/kg). C1 area pressor sites were first identified by microinjection of L-glutamate (100nl, 150mM) and then lesions were created with bilateral microinjections of ibotenic acid (100 nl, 75mM). The following mean arterial pressures (mmHg, mean ± SEM) were observed:

CONTROL 100% N_2 CONTROL NaCN

PRELESION 118±6 141±5 117±5 147±6

POSTLESION 74±6 65±7 77±5 67±6

Stimulation of arterial chemoreceptors by brief periods of hypoxia (100% N_2) or intravenous infusion of NaCN produced marked increases in blood pressure. Following placement of lesions in the C1 pressor area, stimulation of arterial chemoreceptors resulted in depressor responses in all 8 dogs. These results indicate that in the dog, the C1 pressor area of the rostral ventrolateral medulla is the essential brainstem site for expression of the blood pressure response to stimulation of arterial chemoreceptors.

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ABSTRACTS

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INDOOR EXHALED CARBON MONOXIDE (CO) AT HIGH ALTITUDE (HA).

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Exhaled CO, marker of tobacco smoke environmental pollution, could be expected to be low in non smokers at HA. We measured exhaled CO in non smokers lowlanders and in non smokers residents of high altitude villages during a trekking to Pyramid Lab in Khumbu Valley, Nepal. **Subjects:** 6 M lowlanders (3 Italians, 3 Nepalese) 39.8(+8) years = group L; 10 residents at 3540m (5F,5M) 43.2 (+15) yrs=Group H1; 10 residents at 4240m (3F,7M) 32.4, (+9) yrs= Group H2. **Method:** indoor exhaled CO was measured at different altitudes during trekking in L and in their village in H1 and H2. **Results:** mean CO ppm (SD)

	1700m	2800m	3540m	4240m	5050m
A	1,8(1,5)	1,28(1,7)	2,16(1,7)	1,3(1,5)	0,8(1,4)
H1,H2			3,8(1,4)**	3,7(1,5)**	

ANOVA test: ns in L; CO significantly higher ($p<.05$) in H1 and H2 vs L at the same altitude. **Conclusions:** a high domestic pollution is present in HA lodges, probably due to fires and no provision for chimney. The difference between L and H1, H2 is explained by the longer resident's exposure. The fires indoor pollution is also demonstrated by the lower level of CO, even if not significant ($p=.07$) measured in Pyramid Lab, provided with heating panels. Supported by Italian CNR.

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ATMOSPHERIC POLLEN AND SPORES AND INDOOR DOMESTIC MITES AT HIGH ALTITUDE.

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Indoor and outdoor environment plays an important role in respiratory health of asthmatic and rhinitic subjects. **Aim:** to study environmental conditions at HA. With a portable aspirator we collected atmospheric samples (outdoor 6 hours aspiration) and indoor air samples (aspiration near beds and floors) at different altitudes in Khumbu Valley, Nepal. After a technical process, samples were examined by optic microscopy. **Results:** total amount in 300 microscopic fields

	1700m	2800m	3540m	4240m	5050m
gramineae	94	57	22	0	13
urticaceae	32	0	0	0	0
salicaceae	6	0	0	0	0
not identified	5	13	3	6	1
total pollen	137	70	25**	6**	14**
mite	16	7	4	0	0

Conclusions: pollen and mite concentration progressively significantly decrease (ANOVA test $**=p<.05$) but are still present at high altitude (in Alpes area: absence above 3000m) and could induce bronchial and nasal attacks in allergic rhinitic and asthmatic subjects. Supported by Italian CNR

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RESPIRATORY FUNCTION AT INCREASING ALTITUDES IN HEALTHY LOWLANDERS.

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The HA lower air density and increased thoracic fluid can modify the values and the shape of flow volume curve(FV). FVC drop was reported in hypobaric chamber above 5000m (Welsh ARRD 147, 1993). **Aim:** to study FV at increasing altitudes during a trekking in Khumbu Valley, Nepal. 4 low residents M, age 38-50 performed forced expiration (MicroMedical Spirometer) at different altitudes in the morning 24hrs after arrival. Sat%O₂ was measured with pulse oximetry. **Results:** are expressed as percent of baseline. Sat% in absolute value.

Altitude(m)	1700	2800	3540	4240	5050	5050 *
FVC	100	92,5*	92,2*	90,7*	90*	87,2
PEF	100	110,2	121,4*	129,4*	135,2*	133**
FEV1	100	99	102,5	98,2	99,7	99,2
FEF25-75	100	109,5	120,7*	129*	130*	133,7*
MEF25	100	94	92,5*	90,5*	90,3*	89,2*
Sat%O ₂	96,4	94,1	89,7*	88,4*	85,8*	88*

A significant drop of FVC and MEF25 and increase in flows, as expected, were found above 2800m. (* $p>.05$) The stay at the same altitude induces a further drop of FVC. A restrictive change is already present around 3000m probably due to higher lung tissue fluid as showed also by drop of MEF25 Supported by Italian CNR

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VENTILATORY DYNAMICS ALTER WITH ACCLIMATISATION

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Climbers often comment that a constant work rate is important for best performance at high altitude. We have noticed empirically that ventilatory matching at the start of exercise on arriving at a new altitude is less rapid and may result in an unstable, oscillating breathing pattern for a minute or so, this effect disappearing after acclimatisation. These observations suggest that fast, dynamic control of breathing may be important in acclimatisation. This study aimed to assess one aspect of ventilatory dynamics which is known to depend, at least in part on the function of the peripheral chemoreceptors. We compared the time constants of the increase in ventilation at the start of mild to moderate cycling exercise (60W) under four conditions on members of the BMEME 1) At sea level breathing air (control n=40), 2) At sea level breathing 12% oxygen (acute hypoxia n=36), 3) On arrival at 5300m (subacute hypoxia n=46) and 4) After acclimatisation to 5300m (acclimatised n=41). Acute hypoxia at sea level, and also on arrival at 5300m did not show a significantly faster onset of ventilation in exercise than control, with D values of 1) 86.3 (2.3SE) 2) 90.9 (2.8) 3) 91.1 (1.2). Acclimatised subjects did adjust their ventilation faster at the start of exercise, D=94.3 (1.9) p<0.01. This enhancement of ventilatory change at the start of exercise would be consistent with increased peripheral chemoreceptor drive after acclimatisation.

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DYNAMIC CHEMOSENSITIVITY TO CO₂ INCREASES WITH ACCLIMATISATION TO CHRONIC HYPOXIA IN MAN.

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We show elsewhere that dynamic ventilatory changes are altered by acclimatisation. This study examined the changes in ventilation in response to small volumes of carbon dioxide given early (0-300ms), or late (300-600ms) in each inspiration. This dynamic stimulus (a variant of "reverse tube breathing") causes greater increases in ventilation if the stimulus is delivered early, rather than late in inspiration at sea level. The difference between early and late responses increases with exercise. We studied members of the BMEME at steady-state mild-moderate (60W) exercise during acute hypoxia at sea level (12% oxygen, n=29), and after acclimatisation to 5300m (ambient air, n=31). During acute hypoxia the early CO₂ stimulus increased ventilation by 4.3l/min (0.5)(mean (SE)), late CO₂ gave 3.7l/min (0.4). There was no dynamic component (difference) in acute hypoxia. After acclimatisation, however, early stimuli gave increases of 15.6l/min (1.6) c.f. late 10.2l/min (0.9), the difference between early and late responses averaged 5.3l/min (1.1) ($p<0.001$). Further enhancement of dynamic responses was seen in a further group of 6 climbers on return from >7100m on Pumori or Everest. Early stimuli gave 23.3l/min(3.3) increase in ventilation c.f. 8.8l/min(1.5) with late. This dynamic response was greater than that of those acclimatised to 5300m (14.4l/min c.f. 5.3l/min $p<0.025$). The dynamic component of the CO₂ stimulus, attributed to the peripheral chemoreceptors, is markedly enhanced by acclimatisation to hypoxia.

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VENTILATION AND HYPOXIC VENTILATORY RESPONSIVENESS IN FIRST GENERATION CHINESE-TIBETAN RESIDENTS OF 3658 M

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Lifelong Tibetan residents of 3658 m ventilate as much as acclimatized newcomers and have higher hypoxic ventilatory response (HVR) A values than immigrant Han. We measured resting ventilation and ventilatory responses of 21 men born at 3658 m to Tibetan mothers and immigrant Han (Chinese) fathers and compared them to values previously obtained in 27 Tibetan (Tib) and 30 Han residents of 3658 m. The Han-Tibetans (HT) were heavier than Han or Tibetans. Sa_{O₂} (%) and RQ were similar for all three groups but Han-Tibetans had lower end-tidal PCO₂ values than Han or Tibetans, indicating greater alveolar ventilation per unit CO₂ production. Han-Tibetans had lower HVR A values and hypercapnic ventilatory response (HCVR) S values compared with 3658 m Tibetans; instead, their values were similar to 3658 m Han. However, Han-Tibetans were similar to Tibetans in minute ventilation (l/min BTPS). Thus, duration of high-altitude residence rather than parentage appears to make a greater contribution to hypoxic ventilatory chemosensitivity among Han-Tibetan residents of 3658 m.

Group n Weight HVR A HCVR S Ve Pe_{TCO₂} Sa_{O₂}
Tib 27 55.3±1* 121±17* 1.44±.12* 11.5±.5 32.2±.5* 89.3±.3
Han 30 52.8±1* 81±10 1.10±.11* 10.1±.4* 31.6±.5* 90.2±.5
HT 21 59.3±1 64±15 0.82±.13 12.1±.5 29.9±.6 90.9±.7

* $p<0.5$, compared with Han-Tibetans

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ASSESSMENT OF VISUAL FUNCTION AT ALTITUDE USING MOVEMENT DETECTION PERIMETRY

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Objective: To compare visual function at sea level with that at altitude using the detection of movement within the central field of vision.

Method: The central visual field of 62 subjects was studied at sea level and at 5,300 meters using vertical bars moving horizontally on the screen of a laptop computer.

Results: Visibility of moving targets at altitude was reduced compared to sea level values.

Conclusion: Movement Detection Perimetry provides a method for assessing visual function at altitude.

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Changes in Scotopic and Photopic Vision at Altitude

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ABSTRACTS

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PERICYTE-CNS ENDOTHELIAL CELL CROSS TALK DURING HYPOXIA

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Cells of the CNS microvasculature (MV) must cope with changes in their environment such as toxins, metal ions and low oxygen (O_2). The MV response to stress can include activation of DNA binding proteins, induction of transcription factors or immediate early genes (IEGs) and synthesis of stress proteins. We present evidence that hypoxic stress induces rapid changes in CNS pericytes (PC). These changes involve PC activation and alterations in eicosanoid metabolism which precedes endothelial cell (EC) activation. CNS MV isolated from Sprague-Dawley rats were exposed *in vitro* to moderate hypoxia (1%-10%) in the presence of MEM+ glucose at 37°C for varying periods of time. MV remained viable for 72 hours. By 2-4 hours, PC display increased transferrin receptors (tfr) and by 6 hours express MHC Class II as well as a number of adhesion proteins. PC and to a much lesser extent EC reactivity to low O_2 could be duplicated in primary culture and was associated with an early change in PC eicosanoid repertoire. By 6 hours in hypoxic conditions, PC production of PGE was inhibited while synthesis of PGD was enhanced. The PGE to PGD switch was PC-dependent. EC activation in response to low O_2 could be duplicated by the addition of $\Delta 12PGJ$, a metabolite of PGD. $\Delta 12PGJ$ is taken-up by the nucleus and may be involved in gene regulation of stress proteins. PC may communicate to EC during hypoxia via eicosanoids and may be an early sensor to microvascular stress.

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IMMUNE RESPONSE OF RAT AND PIKA TO HYPOXIA

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The immune function of rat and Tibetan plateau native mammal-Pika (*Ochotona curzoniæ*) during hypoxia was studied in simulated altitude chamber for various period. Hypoxia at 5 and 7 Km of altitude for 24 h increase plasma ir-CRF and corticosterone, simultaneously decrease 28% and 41% ($P<0.05$) of T lymphocyte proliferation of rat only respectively in comparison with control of 2 Km, similar suppression of the immune function was found in adrenalectomized (ADX) rats as well, but did not in Pika under above condition. The humoral immunoresponse to sheep red blood cells was also suppressed by hypoxia 7 Km for 10 and 21 days in rats, not in Pikas. In both intact and ADX rats treated with CRF (icv 1 μ g/animal) resulted in a decrease of T lymphocyte proliferation, which was similar to that induced by hypoxia. However treated icv with CRF antiserum in rats, the immunosuppressive effect of acute hypoxia was partly blocked. In the animals treated by icv 5 nM NE at 7 Km, T lymphocyte proliferation declined 29%, treated with α -receptor blocker phenolamine, brought the decline back up partly. These evidences suggest hypoxia suppress the immune function. CRF plays an important role in modulating the immune response to hypoxia stress, the suppressive effect of NE may act through CRF. Adrenal is not markedly involved in the suppression.

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EFFECT OF TEMAZEPAM ON OXYGEN SATURATION DURING SLEEP AT HIGH ALTITUDE.

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This study was conducted on 12 volunteers at 5300m on Mt Everest. Subjects were randomised to have temazepam 10mg or placebo on 2 consecutive nights. Arterial oxygen saturation was measured continuously during the night, using pulse oximetry, and a subjective appraisal of the quality of sleep was also made. Results were analysed with respect to mean oxygen saturation, minimum saturation and the amount of oscillation of saturation overnight. All subjects noted subjective improvement in quality of sleep with temazepam compared to placebo. The response to temazepam differed, depending on the duration of acclimatisation. Subjects who had only recently arrived at altitude 5300m showed a small decrease in the oscillation of the saturation. This agrees with previous studies done in the field. Subjects who had acclimatised over at least 3 weeks showed a similar decrease in oscillation and subjective improvement in sleep. However, the mean saturation showed a consistent increase during sleep with temazepam when compared with the placebo. It is evident that temazepam subjectively improves the quality of sleep without a markedly detrimental effect on arterial oxygenation at 5300m. In addition it appears that in better acclimatised subjects the use of benzodiazepines leads to a small increase in mean saturations. The exact mechanism for this variable response is unclear.

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ANESTHETIC PRACTICE IN HOSPITALS SITUATED AT MODERATE ALTITUDE

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This study was designed to evaluate the anesthetic and critical care practice of 15 hospitals at high altitude (range 4200-11,880ft, average=7569ft). The number of anesthetics administered annually varied from 250-14,512 (average=3374). All of the institutions provided anesthesia for general surgery/orthopedics; 73% for obstetrics; 93% for pediatric cases; 73% for thoracic surgery; and 27% for cardiac surgery. Expected preoperative SaO_2 while patients were breathing room air varied from 87-96%. Reported problems unique to providing anesthesia in altitude regions included: equipment limitations; the problem of ensuring amnesia; frequent perioperative bronchospasm; a bias toward using regional anesthesia; more frequent determination of arterial blood gases; perioperative hypoxemia; and frequent low birth weight neonates. Five institutions reported experience with concurrent altitude disease - four with common AMS in low altitude residents presenting for medical care, and three reporting HAPE in both low altitude residents and obstetrical patients, as well as an increased incidence of HELLP syndrome (a variant of severe pre-eclampsia with hemolysis, elevated liver enzymes, and low platelets). Major surgical procedures and anesthesia can be safely performed at moderate altitude, though the margin of safety with respect to hypoxia would be expected to be smaller in institutions located at significant altitude or in patients with severe anemia or cardiopulmonary disease.

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ADAPTATION TO INTERVAL NORMOBARIC HYPOXIA INCREASES THE ENDURANCE OF PHYSICAL LOADS IN PATIENTS WITH CORONARY HEART DISEASE AND STABLE ANGINA

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69 men 53±2 years old with coronary heart disease (CHD) and stable angina pectoris functional classes (FC) I-IV, (SaO₂ 93% at rest) were examined. 49 of them received the course of interval hypoxic training (IHT), 20 served as controls. The patients of I-II FC did not get any drugs (but nitroglycerin sublingually), the patients of the III-IV FC received traditional antianginal therapy. IHT course consisted of 20-25 daily seances with several series of inhalation 10-11% oxygen in nitrogen followed by breathing in normoxic air. The total time of hypoxic mixture inhalation in one seance was 20-60 min. After the IHT course exercise threshold and the volume of work increased by 25.8%, and by 45%, double product at the exercise threshold increased only by 7.4% and at the first step of the exercise decreased by 13%. 23 patients after the IHT course and only 2 patients of the control group improved their FC of angina. The frequency of angina episodes and their duration (24 h. ECG monitoring) decreased by 64% and 65%, their duration by 70% and 68% correspondingly. According to our data beneficial effect of IHT in CHD patients may be attributed to blood oxygen content increase, to the improvement of erythrocytes and leukocytes filtration and oxygen utilization in the tissues.

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EFFECT OF HIGH ALTITUDE ON LUNG PERfusion MEASURED BY SCINTIGRAPHY

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Hemodynamic hypotheses (overperfusion, capillary stress failure) regarding the pathophysiology of high altitude pulmonary edema (HAPE) are based on the assumption that hypoxic vasoconstriction is inhomogeneous. To examine this assumption we performed perfusion scintigraphy with ^{99m}Tc-MAA in 22 subjects at 550 m and 6 hours after rapid ascent to 4559 m, prior to any manifestation of HAPE. Five subjects had radiographic evidence of HAPE 18 to 36 hours after scintigraphy. At high altitude, the upper part of the lung and the periphery were better perfused in all subjects. Two projections (anterior-posterior) were used for preliminary semiquantitative analysis of the perfusion scans. Each lung was divided into 3 regions (upper-middle-lower) and one point was assigned to each region demonstrating inhomogeneous radionuclide distribution. The total scores (mean ± SE) were:

	All HAPE	Control	p (Mann-Whitney)
(n=22)	(n=5)	(n=17)	
Low altitude 1.1 ± 0.2	1.0 ± 0.5	1.2 ± 0.3	n.s.

4559 m 2.4 ± 0.3 1.6 ± 0.5 2.6 ± 0.4 n.s.

p (Wilcoxon) 0.001 n.s. 0.002

Inhomogeneity involved predominantly the upper lung fields. Regions of the lung where edema developed subsequently did not show exceptional perfusion patterns at the time of the examination. The results show that the lung perfusion becomes more inhomogeneous predominantly in the upper lung areas recruited at high altitude. There are no significant differences between control and HAPE.

Supported by a grant of the Swiss National Fundation.

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SUMMARY GAS PRESSURE (P_{O2}/P_{CO₂}) IN BLOOD PLASMA DURING BREATHING TESTS EXERCISE IN "NORMAL" ADULTS (data for the hypoxia state diagnosis)

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Monitoring for hypoxia state diagnosis, blood tests.

Tests on P_{O2} and P_{CO₂} (mm Hg), SO₂, p_H, etc. are commonly used to study the human effect of hypoxia. Beginning from 1987 we also use tests on gases, gas exchange in the blood plasma. At the same time we have developed non substitute conventional (P_{O2} and P_{CO₂}) tests, which include them as a part choosing only additive characteristic-pressure. The knowledge of "normal" values for these tests are helpful for hypoxia effect evaluation.

RESULTS: We have carried out a series of tests on gases in blood plasma in 100 persons with the diagnosis not supported by categorization, are presented here. The investigations have been carried out during air respiration in supine position.

RESULTS: The values have been taken from 4 journals (A., a-symptom (ad), a-symptom (s), a-symptom (v), a-symptom (u); v-criteria (ad), v-criteria (s), v-criteria (v)).

RESULTS: P_{O2} is from 70 mm Hg and mean P_{O2} is from 90 mm Hg. P_{CO₂} was measured.

RESULTS: P_{O2} mm Hg P_{CO₂} mm Hg p_H mm Hg

A 70 70±0.5 35-60±0.2 128-340±4 7-9±0.005 96-74±0.07

V 70 70±0.4 35-60±0.2 128-340±4 7-9±0.005 96-73±0.06

U 70 70±0.4 35-60±0.2 128-340±4 7-9±0.005 96-73±0.06

v 70 70±0.5 35-60±0.2 128-340±4 7-9±0.005 96-73±0.06

ad 70 70±0.4 35-60±0.2 128-340±4 7-9±0.005 96-73±0.06

s 70 70±0.4 35-60±0.2 128-340±4 7-9±0.005 96-73±0.06

u 70 70±0.4 35-60±0.2 128-340±4 7-9±0.005 96-73±0.06

v 70 70±0.4 35-60±0.2 128-340±4 7-9±0.005 96-73±0.06

RESULTS: P_{O2} mm Hg P_{CO₂} mm Hg p_H mm Hg

vU ad 70 70±0.4 35-60±0.2 128-340±4 7-9±0.005 96-73±0.06

Uv ad 70 70±0.4 35-60±0.2 128-340±4 7-9±0.005 96-73±0.06

UvU ad 70 70±0.4 35-60±0.2 128-340±4 7-9±0.005 96-73±0.06

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UvUvU ad 70 70±0.4 35-60±0.2 128-340±4 7-9±0.005 96-73±0.06

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ABSTRACTS

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BLOOD GASES COMPOSITION IN INTERVILLOUS SPACE

(data for hypoxia states evaluation)

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The data on P_02 , PCO_2 , pH , SO_2 in uterine intervillous space blood are used to evaluate hypoxia states during pregnancy.METHODS: Beginning from 1987 while evaluating human gas exchange and hypoxia effects, we use tests on summary gas pressure (SGP) (P_02 - PCO_2 - pH). These tests neither exclude nor include the arterial partial (P_02), (PCO_2) values, but include them as a part choosing only additive characteristic - pressure. Their use, as we see it carries some additional information.

Analysis of blood gases composition in uterine intervillous space during pregnancy has been performed according to 6 subjects (1987). We used their data for a similar "independent" examination of some characteristics of tests on SGP.

RESULTS: Two groups have been selected and their quantitative analysis is presented.

 P_02 in intervillous space blood more or equal to PCO_2 : 43.38 ± 1.6 36.00 ± 1.2 79.34 ± 2.0 7.445 ± 0.023 P_02 in intervillous space blood less than PCO_2 : 33.00 ± 3.1 46.3 ± 2.0 79.0 ± 2.5 7.390 ± 0.021 The data show that P_02 and PCO_2 can have the wide range of deviations with minimization of SGP variations.DISCUSSION: Hence, taken separately P_02 and PCO_2 values, not always necessary and sufficient for hypoxia shifts in uterine intervillous space blood evaluation during pregnancy.Minimization of SGP in intervillous space blood deviations in P_02 , PCO_2 , pH shifts occurs due to replacement acts between P_02 and PCO_2 . Additional data for tests on summary gas pressure are reported before.

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EFFECT OF INCREASING MAXIMAL HEART RATE ON MAXIMAL O_2 UPTAKE IN RATS ACCLIMATIZED TO SIMULATED ALTITUDE

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The effect of increasing maximal heart rate (H_{max}) by atrial pacing on maximal O_2 uptake ($V_{O_{max}}$) and exercise hemodynamics was investigated in rats acclimatized for 3 weeks to a barometric pressure of ~ 370 Torr; $P_{O_2} \sim 72$ Torr. $V_{O_{max}}$ was determined using an incremental treadmill exercise protocol. One group of 10 rats exercised at $P_{O_2} \sim 72$ Torr, a second group of 10 exercised at $P_{O_2} \sim 142$ Torr. Each rat ran twice; in one run the heart rate was maintained at 600 b/min by atrial pacing, in the other the heart was not paced. The paced (P) and not paced (NP) runs were alternated. In hypoxic exercise, increasing H_{max} by 19%, from 505 ± 7 to 600 b/min, resulted in an 8% increase in $V_{O_{max}}$ (ml(STPD)/(min.kg)), from 55.2 ± 1.2 to 59.6 ± 1.8 ; $p < 0.05$. The increase in $V_{O_{max}}$ with pacing was accompanied by a 10% increase in maximal cardiac output (Q_{max} , ml/(min.kg)) from 454 ± 24 to 500 ± 27 ($p < 0.05$) which occurred in spite of a 9% decrease in maximal stroke volume (SV_{max} , ml/kg) from 0.92 ± 0.05 in NP to 0.84 ± 0.04 in P ($p < 0.05$). There was no effect of pacing in arterial or venous blood O_2 content. A similar pattern was observed in the rats that exercised in normoxia. It is concluded that increasing H_{max} in hypoxia-acclimatized rats results in small but significant increases in Q_{max} and convective O_2 transport which are translated into an increase in $V_{O_{max}}$. The results indicate that acclimatized rats tolerate one bout of exercise at high H_{max} without apparent deleterious effects. Supported by NIH grant HL 39443.

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CNS RESPONSES TO VOLUNTARY HYPERVENTILATION IN HYPOXIC CONDITIONS

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The ordinary regulation of respiration doesn't ensure the effective adaptation to hypoxia. Our aim was to study CNS responses to voluntary hyperventilation (VH) in hypoxic conditions. We examined 43 healthy males at rest and during 2 min VH. PCO_2 and EEG were recorded in normal conditions, during the stepped rise in the decompression chamber up to 7000m and in the mountains of Tian-Shan (up to 4200m, 22 days). In the chamber PCO_2 after VH reduced from 17.6 ± 0.58 (5000 m) to 14.5 ± 0.59 mm Hg (7000m). In the mountains on 12 day at 4100m PCO_2 during VH reduced to 16.0 ± 0.58 mm Hg. In normal conditions we saw the following changes of EEG during VH: activation and disorganization of δ -rhythm, appearance of short groups of θ and Δ waves. These changes were not so expressed during VH in acute hypoxia. At 7000m VH temporarily removed θ and Δ waves, caused by acute hypoxia. On the 12 day in the mountains (4100m) VH was followed by θ and Δ rhythm on EEG of 6 subjects from 10. Querol showed that VH at 3500m didn't cause the expressed changes on EEG of high-altitude residents. These studies demonstrate the phasic adaptation of CNS to hypoxia. First plays role the hypoxic factor, then the sensitivity to hypoxia.

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P-SELECTIN, E-SELECTIN, AND VON WILLEBRAND FACTOR IN ACUTE MOUNTAIN SICKNESS AND HIGH ALTITUDE PULMONARY EDEMA

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Impaired pulmonary gas exchange occurs in acute mountain sickness (AMS), and non-cardiogenic pulmonary edema occurs in high altitude pulmonary edema (HAPE). To determine if pulmonary endothelial cell activation or injury occurs after acute ascent to high altitude, in AMS, or in HAPE, we measured plasma concentrations of E-selectin, P-selectin, and von Willebrand factor (VWF). Selectins are glycoproteins expressed on injured or activated endothelial cells that mediate tethering of leukocytes to injured endothelium and, along with VWF, are secreted into plasma. We measured plasma concentrations of E-selectin, P-selectin, and VWF in seven control subjects at sea level and after ascent to 4200 m on Mt. McKinley, Alaska. We also measured levels of these markers in five climbers with AMS and four climbers with HAPE who presented to a medical camp at 4200 m on Mt. McKinley. E-selectin significantly increased from 5.7 ± 3.5 ng/ml at sea level to 15.6 ± 9.3 ng/ml after ascent to altitude. In the control group, P-selectin and VWF after ascent to altitude were not significantly different than sea level values. In climbers ill with AMS and HAPE levels of selectins E and P were not significantly different than the control group at high altitude. Levels of VWF were increased in climbers with HAPE (156% of control values), but not in climbers with AMS, as compared to controls at altitude. We conclude that an increase in E-selectin after ascent to altitude arises from endothelial cell activation, but no further increase was seen with AMS or HAPE. This contrasts with ARDS where E-selectin and P-selectin plasma concentrations are markedly elevated. The changes in E-selectin with ascent to altitude and increased VWF in HAPE suggest that vascular injury plays a role in high altitude illness.

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EFFECTS OF LOW CARBON DIOXIDE TENSION ON HYPOXIC CEREBRAVASCULAR RESPONSES

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In human at high altitude, the hypoxemia-hypocapnia is a physiological fundamental phenomena. The present study observed the effects of low carbon dioxide on hypoxic cerebrovascular responses in rats and rabbits. The results showed: (1) The two effects of hypoxemia and hypocapnia clearly have opposing effects on cerebral circulation. After lesioning the cholinergic center, substantia innominata (SI), the opposing effect of hypocapnia on hypoxemia still remained. (2) Hypocapnia of various degree have different opposing effects on the increase of CBF caused by hypoxia. A long duration of hypocapnia (2/3 value of normoxia) can keep the hypoxia CBF at normoxia level. (3) In vitro, the low carbon dioxide tension can antagonize the cerebrovascular dilatation induced by hypoxia. Cerebral vessels appear obvious constriction under hypoxia-low carbon dioxide tension. Destroying the endothelial can not abolish this effect. But in Ca^{2+} -free solution, low carbon dioxide tension no longer induced cerebrovascular constriction. These results suggest that the cholinergic center (SI) and endothelial have no evident effect in the effects of low carbon dioxide tension on hypoxic cerebrovascular responses, but H^+ and Ca^{2+} play a noticeable role.

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FLUID DISTRIBUTION AND TISSUE THICKNESS CHANGES IN HIGH ALTITUDE SHIFT WORKERS IN THE ANDES (>3,600 m)

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The mines in Chile are among the highest in the world. Today the personnel of the mines are usually shift workers (SW) having a time schedule of 10 working-days at altitude (AL) >3,600 m and 4 days rest at sea-level (SL). The hypothesis was tested whether fluid shifts from the intra- to the extravascular compartments in SW (N=11, male, age 34 ± 10.3 yrs, body mass (BM) 67.0 ± 10.3 kg, personal history of AL shift working >5 yrs) are altered compared to a control group (CG) (Europeans, N=5, male, age 40.8 ± 5.5 years, BM 82.5 ± 5.6 kg, no shift workers) after transition from SL (380 m) to 3,600 m. Osmolarity (OSM), colloid osmotic pressure (COP), total proteins (PRO), albumin (ALB) and tissue thickness (TT) changes (ultrasound method) at the head and tibia were determined. After transition from SL to AL the SW showed a BM increase to 67.7 ± 10.1 kg ($P<0.01$) whereas in the CG a decrease was observed (81.9 ± 5.4 kg). After 1 week at AL in both groups a decreased BM was found (SW 66.9 ± 10.2 kg ($P=0.05$), CG 80.9 ± 6.3 kg). During the 4 resting days at SL in both groups BM normalized. OSM and PRO did not change, whereas ALB in SW decreased from 5.1 ± 0.3 g \cdot dl $^{-1}$ at SL to 4.9 ± 0.3 g \cdot dl $^{-1}$ after transition to AL ($P=0.05$) and remained decreased ($P<0.01$). The fall in ALB in SW was paralleled by a drop in COP (at SL 30.0 ± 3.2 mm Hg, at AL 27.6 ± 2.5 mm Hg, $P<0.01$). TT in SW increased at the front from 4.3 ± 1.0 mm to 5.0 ± 0.8 mm at AL and remained above control level thereafter ($P<0.01$). The CG showed a similar trend.

Conclusions: Protein shifts from the intra- to the extravascular space lead to fluid accumulations in the interstitial space which might be the first signs of edema formation at AL exposure > 3,600 m.

Supported by grant from the GERMAN SPACE AGENCY (BMFT-DARA)

* All values given here are arithmetic means \pm SD; significance level $P<0.05$.

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TIME COURSE OF ERYTHROPOIETIN IN HIGH ALTITUDE SHIFT WORKERS IN THE ANDES (ATACAMA, CHILE)

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The mines in Chile are among the highest in the world. Today the personnel of the mines are usually shift workers (SW) having a time schedule of 10 working-days at altitude (AL) >3,600 m and 4 days rest at sea-level (SL). The hypothesis was tested whether the response of the erythropoietic system by means of erythropoietin (EPO) production and release in SW (N=11, male, age 34 ± 10.3 yrs, body mass (BM) 67.0 ± 10.3 kg, personal history of AL shift working >5 yrs) is altered compared to a control group (CG) (Europeans, N=5, male, age 40.8 ± 5.5 years, BM 82.5 ± 5.6 kg, no personal history of AL shift working) after rapid transition from SL (380 m) to 3,600 m AL and vice versa. At SL the SW showed a mean EPO level of 5.2 ± 2.4 mU \cdot ml $^{-1}$, CG 8.2 ± 2.5 mU \cdot ml $^{-1}$. After 2 days at AL the EPO level in SW increased significantly to 24.2 ± 11.2 mU \cdot ml $^{-1}$ ($P<0.001$). CG showed a similar trend (29.6 ± 6.2 mU \cdot ml $^{-1}$). During the week at AL the EPO levels of SW decreased significantly (15.5 ± 4.2 mU \cdot ml $^{-1}$, $P<0.001$) and in the CG as well (16.1 ± 5.1 mU \cdot ml $^{-1}$). After 4 days at SL SW approached their control level (5.2 ± 2.9 mU \cdot ml $^{-1}$) whereas CG showed decreased EPO concentrations compared to their controls (4.1 ± 1.2 mU \cdot ml $^{-1}$). Pre transition to AL hemoglobin (HB) in SW were higher (11.0 ± 0.7 mmol \cdot l $^{-1}$) than in the CG (10.3 ± 0.7 mmol \cdot l $^{-1}$). During AL SW showed a HB decrease (10.0 ± 0.7 mmol \cdot l $^{-1}$, $P<0.001$) which was not seen in CG (10.4 ± 0.6 mmol \cdot l $^{-1}$). Still 4 days at SL SW showed a prolonged HB decrease (9.6 ± 1.0 mmol \cdot l $^{-1}$, $P<0.001$), the CG followed this trend (9.7 ± 0.8 mmol \cdot l $^{-1}$).

Conclusions: The hypothesis has to be refuted that EPO response to a hypoxic stimulus (>3,600 m) is altered in Chilean miners which shades new light upon the reasons of excessive erythrocytosis at AL.

Supported by grant from the GERMAN SPACE AGENCY (BMFT-DARA)

* All values given here are arithmetic means \pm SD; significance level $P<0.05$.

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THE ROLE OF GLUTAMATE IN VENTILATORY RESPONSES TO MODERATE HYPOXIA

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The present study tests the hypothesis that NMDA-type glutamatergic processes are involved in generating the ventilatory response to moderate hypoxia. Ventilation was monitored in lightly anesthetized (Urethane 1g/kg) golden-mantled ground squirrels during exposure to air, 10-12% O₂ and 4-5% CO₂. Observations were repeated following the systemic administration of Dizocilpine (MK-801), a potent non-competitive antagonist of NMDA-type glutamate (GLU) receptors. This species responded to moderate hypoxia with a two-fold increase in breathing frequency (f_r) and slight reduction in tidal volume (V_T). Dizocilpine treatment itself resulted in a 2.5 fold increase in f_r, but had no effect on V_T. Hypoxia following Dizocilpine had no effect on ventilation. Hypercapnia before Dizocilpine increased both f_r (1.6 x) and V_T (2.5 x) but following Dizocilpine it produced only an increase in V_T (2.2 x). These data indicate that GLU, acting via NMDA receptors, plays a role in the generation of both resting breathing and the ventilatory response to moderate hypoxia. Specifically, at rest GLU 1) prolongs inspiration and expiration, 2) reduces respiratory drive, and 3) has no effect on V_T. The absence of any further increase in f_r due either to hypoxia or hypercapnia following Dizocilpine suggests that GLU receptors form part of the pathway involved in eliciting the ventilatory frequency response at some level. This research was supported by the NSERC of Canada.

ABSTRACTS

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LACTATE RELEASE FROM RAINBOW TROUT WHITE MUSCLE CELL: PASSIVE DIFFUSION OR CARRIER-MEDIATED TRANSPORT?

Y.X. Wang, C. F. Misiaszek, G.J.F. Heigenhauser, and C.M. Wood. Dept. of Biology and Medicine, McMaster Univ. Hamilton, Ont., Canada, L8S 4K1. An isolated perfused tail-trunk preparation was used to examine the release of Lac⁻ from post-exercised white muscle. The transmembrane pH gradient was manipulated by varying perfuse pH (approx. 8.4, 7.9, and 7.4) while maintaining P_{CO_2} . The electrical gradient (E_m) was changed by increasing perfuse K^+ from 3mM to 15mM. Transmembrane Lac⁻ distribution is neither pH nor E_m dependent. This suggests that the membrane is very impermeable to Lac and carrier-mediated Lac transport could be involved. Based on this finding, specific blockers: cinnamic acid (CIN), SITS and amiloride were used to identify the potential role of various ion transporters in lactate transport. CIN, a blocker of both Lac/H⁺ co-transporter and Lac/HCO₃⁻, Cl⁻ exchange, significantly reduced Lac efflux while SITS, a more specific blocker for Lac/HCO₃⁻, Cl⁻ exchange, did not show any significant effect on Lac⁻ efflux. This suggests that Lac/H⁺ co-transport is involved in Lac efflux. (Supported by NSERC).

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FUEL USE DURING MAXIMAL INTERMITTENT EXERCISE

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We studied fuel use during maximal short-duration intermittent exercise. Seven males completed three 30-s bouts of maximal isokinetic cycling at 100 rpm, separated by 4 min rest. Total work in bout 1 was 19.3 \pm 0.9 kJ, decreasing to 16.3 \pm 1.0 kJ and 14.2 \pm 1.22 kJ in bouts 2 and 3, respectively. Biopsies of vastus lateralis were taken before and after each bout; VO_2 was measured. Proportions of total fuel use were calculated from VO_2 changes in glycogen (Glyc), PCr, and lactate, and PDH activity for each bout. Fat use was estimated from the difference between total VO_2 and the flux through PDH.

Bout	Anaerobic		Aerobic	
	PCr	Glyc	Glyc	Fat
1	11%	50%	22%	17%
2	11%	46%	21%	22%
3	13%	22%	32%	33%

In bout 1, anaerobic sources accounted for 61% of ATP production, falling to 35% in bout 3; use of PCr (11-13%) was similar in the three bouts, but energy derived from glycogen fell from bout 1 to bout 3. There was a greater reliance on aerobic sources for energy in bout 3 compared to bout 1, but the proportion of fat and glycogen used was similar in all bouts.

* MRC Student; supported by MRC Canada.

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ALTERED BLOOD SUBSTRATE USE IN HUMANS AFTER SHORT-TERM AEROBIC TRAINING

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We examined glucose (GLU) and FFA uptake, and lactate (La) release in 7 subjects before and after 2 hr of daily training for 7 days on a cycle ergometer at 60% $VO_{2\max}$. Subjects underwent both pre- and post-training exercise challenges: a rest period followed by 15 min of cycling at 30%, 65% and 75% $VO_{2\max}$. Leg blood flow was measured, and brachial arterial and femoral venous blood samples were drawn at steady state during each stage. After short-term aerobic training, GLU uptake increased from 0.88 \pm 0.14 to 1.47 \pm 0.21 mmol/min at 65% $VO_{2\max}$. Pre-training La release was 1.68 \pm 0.61 mmol/min at 65% $VO_{2\max}$ and became an uptake (0.25 \pm 0.25) after training. At 75% $VO_{2\max}$, La release decreased from 1.99 \pm 1.27 to 0.41 \pm 0.55 after training. FFA release (0.17 \pm 0.08) became uptake (0.04 \pm 0.06) at 30% and at 75% $VO_{2\max}$, uptake decreased from 0.14 \pm 0.06 to 0.02 \pm 0.07 after training. This study showed increased GLU and La uptake at an intensity commensurate with the La production threshold and attenuation of La release above the threshold. Attenuation of the La response after training was due in part to greater La oxidation in the later stages of exercise, removing circulating La generated in the early phase of exercise. Lower FFA uptake after training is consistent with a decreased sympathetic response. The metabolic benefits appear to be improved GLU utilization, increased La oxidation and conservation of plasma FFA.

*, MRC Student; supported by MRC Canada.

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MUSCLE LACTATE PRODUCTION DURING MAXIMAL EXERCISE IS NOT DUE TO INSUFFICIENT O₂ AVAILABILITY

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Lactate (La) accumulation in contracting muscle in normoxia has traditionally been attributed to insufficient O₂ supply. We examined mitochondrial redox state and La production in human vastus lateralis during maximal exercise. Seven subjects completed 3 consecutive 30-s bouts of maximal isokinetic cycling at 100 rpm, separated by 4 min of rest. Total work in bout 1 was 19.3 \pm 0.9 kJ, decreasing to 16.3 \pm 1.0 kJ and 14.2 \pm 1.22 kJ in bouts 2 and 3, respectively. Biopsies were taken before and after each bout. Muscle La increased in each bout, from 6.6 \pm 0.80 to 8.9 \pm 12.53 in bout 1, from 51.3 \pm 9.24 to 130.8 \pm 14.86 in bout 2, and from 81.7 \pm 10.11 to 106.6 \pm 11.07 in bout 3. Mitochondrial NAD/NADH increased in all bouts; in bout 1, from 0.05 \pm 0.012 to 0.13 \pm 0.029; in bout 2, 0.09 \pm 0.026 to 0.23 \pm 0.046; and in bout 3, 0.20 \pm 0.028 to 0.26 \pm 0.059. La accumulation occurring in the face of elevated mitochondrial oxidation state, indicated that O₂ availability was not limiting for pyruvate oxidation. La production was due to a greater pyruvate production than oxidation, since glycolytic flux was 19-fold greater than PDH activity in bouts 1 and 2, and 6-fold greater in bout 3. Thus muscle lactate accumulation during maximal exercise in humans is due to a rate limitation of pyruvate flux through PDH rather than limited oxygen supply and a fall in mitochondrial oxidation state.

* MRC Student; supported by MRC Canada.

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³¹P MAGNETIC RESONANCE SPECTROSCOPY (MRS) OF THE SHERPA HEART: A PCR/ATP SIGNATURE OF METABOLIC DEFENSE AGAINST HYPOBARIC HYPOXIA

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Of all humans thus far studied, Sherpas are considered by many high altitude biomedical scientists as most exquisitely adapted for life under continuous hypobaric hypoxia. Little is known, however, about how the heart is protected during chronic hypoxia. Hypoxia defense mechanisms in the Sherpa heart were explored by *in vivo*, noninvasive ³¹P MRS. The Sherpas were examined at 21 and 11% FiO₂ on arrival at low altitude study sites and again four weeks later. The concentration ratios of phosphocreatine (PCr) to adenosine triphosphate (ATP) were maintained at steady state values that were about 1/2 those typically found in normoxic lowlanders. Under acute hypoxia the heart rate increased by 20 beats/min from resting values of about 70 beats/min and SaO₂ decreased to about 75%. These perturbations did not alter the PCr/ATP concentration ratios, which remained at about 50% of the values expected in healthy lowlanders. As the creatine phosphokinase reaction functions essentially at equilibrium, these steady state PCr/ATP ratios presumably coincided with about 3-fold higher free adenosine diphosphate (ADP) concentrations. Higher ADP concentrations (i.e. lower [PCr]/[ATP]) were interpreted to correlate with the K_m values for ADP-requiring kinases of glycolysis and to reflect elevated carbohydrate contributions to heart energy needs. This metabolic organization is considered advantageous in hypobaric because the ATP yield/O₂ is 25-60% higher with glucose than with free fatty acids (the usual fuels utilized in the human heart in postfasting conditions).

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ALTITUDE PULMONARY EDEMA IN A SKI RESORT

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A review of medical records of 150 patients with HAPE seen over a 39 month period in a Colorado ski area at 2,928 m revealed the following characteristics: mean age was 34.3 years, 84% were males. Mean time of the onset of symptoms was 3±1.3 days after arrival. Common symptoms were dyspnea, cough, headache, chest congestion, nausea, fever and weakness. Orthopnea, hemoptysis and vomiting were rare occurring in 7, 6 and 1% respectively. Symptoms of cerebral edema occurred in 14%. The mean oral temperature was 99 ± 1.5° and 20% had a temperature exceeding 100°F. The mean systolic blood pressure was 132±20 mm Hg and 17% had a pressure ≥150 mm Hg. Blood pressure was higher in patients >50 years - (142 mm Hg). Rales were present in 85% and were most commonly bilateral or only on the right. A pulmonary infiltrate was present in 88% and was most frequently bilateral (53%) or on the right (34%). The higher incidence of HAPE in men suggests an etiologic role of comparative hypoventilation. The high prevalence of hypertension suggests a high degree of sympathetic stimulation.

The Colorado Altitude Research Institute supported this study.

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SERUM PHOSPHOLIPID BOUND ARACHIDONIC ACID (22:4) AT ALTITUDE AND IN ACUTE MOUNTAIN SICKNESS

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The underlying mechanism of AMS is unknown. The ubiquitous leaky membranes seen in HACE, HAPE, microalbuminuria, and peripheral oedema might be the result of an hypoxic endothelial stress injury, mediated via vasoactive prostanoids (derived from arachidonic acid). 24 subjects (22 male, 2 female, aged 22-64 years) were studied prior to ascent, at 4,120m and 5,200m. Overnight fasting serum samples were frozen at -196°C, stored and assayed by GLC for phospholipid bound free fatty acids. Serum phospholipid bound arachidonic acid (22:4) rose from 11.4(1.8) [mean(SD)] at sea level to 13.6(2.1) at 4,120m and 13.0(1.9); sea level vs 4,120m P<0.001, sea level vs 5,200m P<0.001 (Student T Test). AMS was assessed by a recognized clinical scoring system. There was a weakly positive correlation between an individuals AMS score and the rise in phospholipid bound arachidonic acid ($r^2=0.28$, $P<0.007$). There was no correlation between change in phospholipid bound arachidonic acid and PO₂. This study supports the proposed hypothesis that AMS is an hypoxic endothelial stress injury mediated by prostanoids.

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PLASMA RENIN ADAPTATION TO CHRONIC INTERMITTENT HYPOBARIC HYPOXIA IN WORKERS

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Previous measurements of the plasma renin activity (PRA) in workers adapted to chronic intermittent hypobaric hypoxia have shown a reduction of PRA on the first day at high altitude compared to sea level. In the present study we have evaluated modifications of PRA in a similar population during one regular shift at high altitude. Fourteen normal subjects working on shifts for > 6 months (18 days at 4500 m followed by 10 days below 3000 m) were studied during one of their stays at 4500 m, both on the first and on their ninth or tenth day. Arterial oxygen saturation (O₂ sat), plasma aldosterone (ALDO), fractional excretion of sodium (FENa) and systolic pulmonary artery pressure (PAPs) were simultaneously measured (mean ± SD):

	day 1	day 9 or 10
Ox sat (%)	89.2 ± 3.3	91.4 ± 3.5 *
PRA (ng/ml/hr)	1.3 ± 1.1	2.6 ± 0.9 *
ALDO (ng/dl)	3.9 ± 1.6	4.6 ± 1.5 ns
FENa (%)	60 ± 30	96 ± 46 ns
PAPs (mm Hg)	34 ± 11	27 ± 9 ns

* p = < 0.05 vs day 1; ns = not significant.
A negative correlation between PRA and PAPs was observed ($r = -0.57$, $p < 0.01$), but not among the other variables. These results demonstrate that PRA increases as long as these adapted workers remain at high altitude and is associated with PAPs, a possible marker of adaptation to this environment.

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ABSTRACTS

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AGE AND EXERCISE CAPACITY IN ADAPTED WORKERS
UNDER CHRONIC INTERMITTENT HYPOBARIC HYPOXIA

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Working at different ages under chronic intermittent hypobaric hypoxia (IHH) is rather common at the Andes. This work was designed to evaluate submaximal (6 minutes, submax) and maximal (max) exercise performance as a function of age in 72 adapted, non native male workers exposed to IHH. Exercise was on a treadmill (Bruce protocol) on the first day of their stay at 4500 m (Choquecimpio or Collahuasi mines). Measured variables were heart rate (HR, bpm), systolic BP (SBP, mm Hg) and transcutaneous arterial oxygen saturation (Ox sat, %). Results were (mean \pm SD):

Age group (yrs)	20-29	30-39	40-49
n	15	38	19
duration (sec)	786 \pm 120	737 \pm 146	672 \pm 143*
submax HR	137 \pm 28	132 \pm 28	138 \pm 22
SBP	133 \pm 14	137 \pm 17	149 \pm 25*
Ox sat	82 \pm 7	83 \pm 6	84 \pm 6
max HR	172 \pm 12	168 \pm 15	161 \pm 18*
SBP	148 \pm 14	151 \pm 16	161 \pm 23**
Ox sat	88 \pm 5	81 \pm 6	83 \pm 6

* p < 0.05 vs group 20-29, ** p < 0.05 vs group 30-39. Maximal exercise capacity decreased mildly after 39 years. Workers from 20-39 years old behaved almost identically. Max HR was reduced in the 40-49 group. These results may be used as a reference for adaptation to aerobic exercise in different ages under conditions of significant IHH.

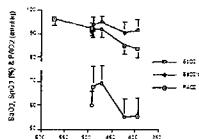
Supported by Minera Doña Inés de Collahuasi.

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RESPIRATORY OBSERVATIONS ON TREKKERS WITH THE 40TH ANNIVERSARY BRITISH EVEREST EXPEDITION

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The expedition provided the opportunity to study the respiratory parameters of a large group of trekkers (11 F, 28 M) as they walked from Jin (1860 m) to Base Camp, Everest (5550 m) during a 3 week period with acclimatisation stops. We have measured FVC, SpO₂ and Pa_{VO₂}. Pa_{BO₂} and Pa_{lV} O₂ (maximum expiratory manoeuvre) were measured using a Teledyne R17 fuel cell. A pulse oximeter (PneuPAC) was used for SpO₂. Oxygen saturation (Sa_{O₂} (calc)) was also predicted assuming PaO₂ to be equal to Pa_{lV} O₂ using an algorithm (Mohai et al. (1977)) in which PaCO₂ (R.Q. = 0.85) was calculated and arterial pH was predicted (Dill et al., (1937), West et al., (1962)). The results demonstrate no difference with gender or age in the progress of acclimatisation in the face of increasing altitude and failed to predict the one case of HAPE. The observed SpO₂ was increasingly unstable over 3440 m. This suggests instability either of ventilation or of V/Q matching. The discrepancy between SpO₂ and predicted Sa_{O₂} (calc) (p > 0.0001 for the higher altitudes) may indicate a deterioration in pulmonary function caused by pulmonary oedema.



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ROLE OF OXYGEN-DERIVED FREE RADICALS AND TISSUE GLUTATHIONE IN EXPERIMENTAL ACUTE GASTRIC MUCOSAL LESIONS INDUCED BY HYPOXIA IN RATS

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The purposes of the present study are twofold. Firstly, to elucidate role of oxygen-derived free radicals in the pathogenesis of acute gastric mucosal lesions induced by hypoxia. Gastric mucosal thiobarbituric acid (TBA) reactants were measured in 10%O₂ hypoxia group of rats. Secondly, to clarify whether gastric mucosal glutathione is involved in the pathogenesis of these lesions or not. Concentration of gastric mucosal glutathione was estimated in 10%O₂ hypoxia group of rats. Rats in 10%O₂ hypoxia group were restrained in stress cages in hypoxia chamber circulated with 10%O₂-90%N₂ mixed gas. Rats restrained in stress cages in room air served as control. Results: TBA reactants increased in both hypoxia and control group with no significant difference between two groups. Gastric mucosal glutathione concentration decreased in both hypoxia and control group. Hypoxia group showed a significantly low value compared with control group. Conclusions: It was suggested that decreased gastric mucosal glutathione play role in the pathogenesis of acute gastric mucosal lesions induced by hypoxia.

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MUSCLE ULTRASTRUCTURE AND BIOCHEMISTRY OF FIRST GENERATION LOWLAND TIBETANS

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We compared in Kathmandu (1300 m), Tibetan resident males (T), offspring of refugees from Tibet, to age and sex matched locals of lowland ethnicity (N). $\dot{V}O_{2\text{max}}$ was similar (37.0 \pm 1.1 [T, n=20] vs. 36.7 \pm 1.1 ml.min $^{-1}$.kg $^{-1}$ [N, n=21] ($\bar{x}\pm\text{SEM}$)). T had a greater ventilatory response to exercise. Maximum blood lactate (11.4 \pm 0.4 [T] vs. 12.3 \pm 0.4 mM [N]) was similar. Muscle biopsy analysis, (8 T and 8 N) showed similar fiber type distribution (type I: 57 \pm 3.4% [T] vs. 58.6 \pm 3.4% [N], IIa: 22.3 \pm 2.9% vs. 24.1 \pm 3.5%, IIb: 15.9 \pm 2.9% vs. 17.4 \pm 1.4%). T tended to have smaller fiber cross-sectional areas than N (3413 \pm 677 [T] vs. 3895 \pm 447 μm^2 [N], P<0.074), but similar number of capillaries per fiber (1.35 \pm 0.23 [T] vs. 1.46 \pm 0.23 [N]) and per muscle fiber cross-sectional area (399 \pm 29 [T] vs. 382 \pm 65 mm^2 [N]). Despite similar $\dot{V}O_{2\text{max}}$, T had a much lower mitochondrial volume density (3.99 \pm 0.17 [T] vs. 5.51 \pm 0.19 % [N], P<0.025) accompanied by proportionally lower activities of citrate synthase and 3-hydroxy acyl CoA dehydrogenase. Activities of lactate dehydrogenase and hexokinase were the same. Tibetans never exposed to high altitude, like their altitude counterparts, are characterised by lower mitochondrial volume to specific $\dot{V}O_{2\text{max}}$ ratio than lowlanders. Like the increased ventilatory response to exercise, this is probably an inborn feature of Tibetans as it still prevails in the first generation lowland offspring. The present data suggest that lowland Tibetans seem to be genetically predisposed to their usual hypoxic habitat.

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RESPONSE OF THE LESSER SPEAR-NOSED BAT TO HYPOXIA: A SMALL MAMMAL WITH AN EXCEPTIONAL TOLERANCE OF LOW OXYGEN

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Per cent change in ventilation in response to 12% inspired O₂ scales with body mass to the -0.27 power in mammals (Boggs and Tenney, *Respir. Physiol.* 58:245-251, 1984). A greater ventilatory response to hypoxia in smaller mammals is a reasonable correlate of their elevated weight-specific metabolism. Another explanation for the systematic relationship between hypoxic ventilatory response and body size in mammals is the relationship between body size and hemoglobin affinity. Lesser spear-nosed bats provide an ideal test of these two alternatives because they are small (43 g), yet have a high affinity hemoglobin (P_{50} of 29). Ventilation in this bat was unaffected by hypoxia except when breathing 8% O₂. However, metabolic rate was significantly depressed at both 10% and 8% inspired O₂. Their hypometabolic response to hypoxia is typical of many small mammals, but was reduced relative to that observed in other small species. The hypoxic ventilatory response of these bats does not support the prediction of a larger hypoxic ventilatory response in small mammals even if expressed as $\Delta V/V_0$. It does support the postulate that an animal with a high affinity hemoglobin will have a lower threshold to its hypoxic ventilatory response regardless of size.

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EFFECTS OF ACUTE AND CHRONIC HYPOXIA ON ATRIAL NATRIURETIC PEPTIDE (ANP) RELEASE FROM CULTURED CARDIOMYOCYTES

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Mechanisms responsible for the increase in plasma ANP levels during acute and chronic hypoxia have not been well defined. We hypothesized that hypoxia increases cardiac ANP release independent of hypoxia-induced elevations in pulmonary artery or right heart pressure. To test this hypothesis, we measured ANP release from primary cultures of neonatal rat atrial and ventricular cardiomyocytes during acute and chronic hypoxia. ANP release is expressed as ng/hr. Values are mean \pm SE, n=3-6 per time point.

O ₂ Conc.	15 min	45 min	24 hrs	48 hrs
A-Normoxia	1.9 \pm 0.4	1.3 \pm 0.2	1.4 \pm 0.2	1.5 \pm 0.2
A-3% O ₂	1.3 \pm 0.1	1.5 \pm 0.1	0.9 \pm 0.6*	0.7 \pm 0.7*
A-Normoxia	0.3 \pm 0.1	0.2 \pm 0.02	0.8 \pm 0.1	1.5 \pm 0.2
A-7% O ₂	0.4 \pm 0.04	0.3 \pm 0.03	0.4 \pm 0.03*	0.4 \pm 0.02*
V-Normoxia	2.7 \pm 0.2	2.6 \pm 0.3	1.6 \pm 0.2	1.9 \pm 0.2
V-3% O ₂	2.7 \pm 0.7	3.0 \pm 0.2	1.0 \pm 0.1*	0.5 \pm 0.06*
V-Normoxia	0.4 \pm 0.02	0.4 \pm 0.02	1.0 \pm 0.1	1.0 \pm 0.1
V-7% O ₂	0.6 \pm 0.7*	1.0 \pm 0.2*	0.9 \pm 0.1	0.8 \pm 0.06

A-atria, V-ventricles, 3% O₂, 7% O₂, and Normoxia = 3, 7, or 21% O₂, 5% CO₂, balance N₂, respectively, * = p < 0.05 vs Normoxia.

We conclude that in atrial and ventricular cardiomyocytes, hypoxia may increase ANP release acutely, but has no effect on or suppresses ANP release after 24 hours.

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LOW PULMONARY DIFFUSION CAPACITY IN SUBJECTS WITH ACUTE MOUNTAIN SICKNESS

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To examine the role of the pulmonary gas exchange in pathogenesis of acute mountain sickness (AMS), we measured the pulmonary diffusion capacity for carbon monoxide (DL_{CO}), arterial blood gas tensions, and the static lung volumes in 32 moderate-altitude (2,260m) residents (24 male and 8 female) after ascent to altitude of 4,700m, in Qinghai Province, China. Twelve of them (10 male and 2 female) had developed AMS by the second day after arrival at the altitude of 4,700m, without any evidence of frank pulmonary edema. In the non-AMS group, all subjects exhibited an increase in DL_{CO} at high altitude (36.2 ± 1.7 ml/min/Torr at baseline vs 47.1 ± 2.5 ml/min/Torr at 4,700m, p<0.01). On the other hand, less marked increase in DL_{CO} was observed in the AMS group (34.7 ± 2.4 ml/min/Torr at baseline vs 35.7 ± 2.2 ml/min/Torr at 4,700m, NS); in four of the twelve subjects with AMS who showed extremely marked symptoms and hypoxemia at 4,700m, the DL_{CO} at the high altitude showed a decrease compared to that at baseline. The AMS group also showed significantly lower vital capacity and Pa_{O_2} at 4,700m compared with the non-AMS group. There was a positive correlation between DL_{CO} and Pa_{O_2} at 4,700m in the AMS group ($r=0.75$, $p<0.01$). These results support the hypothesis that abnormal pulmonary gas exchange in AMS is, at least in part, due to subclinical interstitial edema of the lung.

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2,3-DPG AND ANP IN PATIENTS WITH CHRONIC MOUNTAIN SICKNESS

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To investigate the mechanism of chronic mountain sickness (CMS), we measured the red cell 2,3-diphosphoglycerate (2,3-DPG/RBC), atrial natriuretic peptide (ANP) and arterial blood gases in 18 normal Hans (Non-CMS) and 13 patients (9 Hans and 4 Tibetans) with CMS at an altitude of 4,300m. Hans of both groups were born at or near sea level in lowland and had migrated to high altitude for 14.5 \pm 8.4 yrs in Non-CMS and for 14.4 \pm 7.6 yrs in CMS. Four Tibetans of CMS were born at lower altitude in Qinghai and had lived at 4,300m for the preceding 20 yrs. The data are as follows:

	Non-CMS	CMS
2,3-DPG/RBC, μ M/ml	4.40 \pm 0.13	5.23 \pm 0.16**
ANP, pg/ml	87.60 \pm 4.72	113.41 \pm 5.50**
Hb, g/dl	17.29 \pm 0.41	22.10 \pm 0.58**
Hct, %	59.63 \pm 1.20	68.98 \pm 0.84*
pH	7.442 \pm 0.003	7.377 \pm 0.008**
Pa _{CO₂} , Torr	24.02 \pm 0.27	26.50 \pm 0.67*
Pa _{O₂} , Torr	53.08 \pm 0.72	47.34 \pm 0.98**
A-ABD ₂ , Torr	3.77 \pm 0.41	6.49 \pm 1.01**

Values are expressed as means \pm SE.

* p<0.05 and ** p<0.01 from Non-CMS.

These results suggest that (1) the development of excessive polycythemia at altitude may be in part due to an overproduction of DPG; (2) higher levels of the ANP in patients with CMS could be associated with the pulmonary hypertension.

ABSTRACTS

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Hypoxia in space medicine

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Studying of hypoxia in space medicine is conditioned by the fact that effects of various factors of space flight such as changed gas mixture, overloads, weightlessness, orthostatic instability, and rehabilitation required a clear estimation of oxygen regime and hypoxia in tissues of the organism. Space medicine pioneered in the world in development of an original method for studying the oxygen regime directly in tissues; a distinct decrease in PO_2 has been demonstrated in weightlessness and after returning to the Earth. For the first time new regularities were established, in particular, an inversion of gas exchange and a peculiar reoxygenation of the organism during rapid elevations to an altitude of 12 km, when arterial PO_2 falls quicker and to a greater extent than venous PO_2 . For the first time a fact was established of abrupt cerebral hypoxia during intensive hyperventilation and arterial hyperoxia. Further the efficiency was thoroughly studied of various types of adaptation to hypoxia in mountains, altitude chamber and in breathing gas mixtures including its use in preparation of mountain-climbers to the Everest ascent. A so called "impulse" or interval principle of adaptation has proved to be quite efficient. Such adaptation was produced either in altitude chambers or in normobaric conditions using breathing a gas mixture with 10% O_2 for 5 min 12 times a day simultaneously with exercise. It has been possible to increase the maximal O_2 consumption (MOC) by 9.1 ml/min in 5 days of training. In this process the maximal working power was increased by 23.5% and the total volume of work by 55%. Thus the principle of normobaric interval hypoxia proved to be very efficient in space medicine (Kovalenko E.A., 1989, 1990, 1993). It is extremely important that a similar principle of interval hypoxia but produced with special devices of a firm "Hypoxia Medical" was efficient in the treatment of a broad range of most various diseases (Tkatchouk E.N., 1987-1993; Ehrenbourg I.V., Gorbatchenkov A.A., 1993)

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NUCLEATE VS. ENUCLEATE RED BLOOD CELLS: RATE OF ACID ACCUMULATION IN WHOLE BLOOD

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This study was designed to compare differential abilities of Muscovy Duck and Human blood to adjust to low PCO_2 as a mimic of respiratory alkalosis. We hypothesized that the P_{50} of the bird blood would increase faster than that of the mammal blood. O_2 saturation, [Hb], PO_2 , PCO_2 , pH, and [glucose] of whole blood were monitored for 8 hours after tonometer gas composition was changed from 510, 33, 47, and 40 mmHg partial pressure for N_2 , O_2 , H_2O , and CO_2 , respectively to 534, 33, 47, and 16. Glucose was produced twice during the experiment to approximately maintain starting levels. The duck and human blood had pHs of 7.442 and 7.428 (± 0.03), respectively just before the gas composition change and 7.685 and 7.611 30 min. afterward. Over the ensuing 8 hours, the human blood pH dropped to 7.350 with a base excess of -15 mEq/l while the pH of duck blood dropped to only 7.581, with a base excess of -7. Presumably, glycolysis end products accumulating because of the absence of mitochondria in the RBCs caused the more rapid acidification of the human blood. There was no statistically significant change in P_{50} s calculated at pH 7.4 and 37°C.

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TRAINING AT MODERATE ALTITUDE INCREASES BLOOD OXYGEN CONTENT, MAXIMAL OXYGEN UPTAKE, & RUNNING PERFORMANCE

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Altitude (ALT) training may improve sea level (SL) performance. However the specific mechanisms for this adaptation are unclear. We hypothesized that ALT acclimatization increases blood O_2 content which increases $VO_{2\text{max}}$ and running performance in already well trained athletes. We studied 13 competitive runners (9M, 4F) after: 1) a 2 week lead-in phase of supervised training; 2) 4 weeks of SL training (SL control); 3) 4 weeks of living at altitude (2500m) and training at either 1250m (Hi-Lo) or 2700m (Hi-Hi). All runners received high dose oral iron supplementation to maintain iron stores. We measured: performance (5000m time trial and supramaximal uphill treadmill run); $VO_{2\text{max}}$ (Douglas bags, incremental treadmill run); submax HR, $VO_{2\text{max}}$, cardiac output (C_6H_6 rebreathing), capillary lactate, and DLCO during steady state flat running ($M=10\text{ mph}$, $F=9\text{ mph}$); plasma volume (PV, Evans Blue), HCT, Hgb, blood volume (BV), red cell volume ($RCV=BV \cdot PV$); erythropoietin (EPO); and SaO_2 (pulse oximetry) during base training at simulated ALT in a hypobaric chamber at 1250 and 2700m. **Results:** After 2 weeks of supervised training, 4 weeks of SL training did not increase EPO, Hgb, PV, RCV, or $VO_{2\text{max}}$. 5K time improved by 13.4 sec; submax HR and LAC decreased after SL training. With acute simulated ALT, SaO_2 fell at rest from 98.1% at SL to 94.2% at 2700m and to 92.0±1.7% (1250m) & 79.6±3.1% (2700m) during base training. With chronic ALT (2500m), EPO increased within 48 hrs from 15.8±5.8 to 22.9±5.7, but returned to baseline after 4 weeks (14.0±6.0), and was reduced below baseline after return to SL (12.9±8.1). Hgb (10.3%), HCT (12.7%) and RCV (10.7%) all increased significantly after ALT. $VO_{2\text{max}}$ increased by 5.4% after ALT. 5K time fell an additional 20 sec; this decrease tended to be greater in the Hi-Lo (28 ± 7 sec) compared to Hi-Hi (7 ± 13 sec). 56% of the variance in improvement in 5K time could be explained by the combination of increase in $VO_{2\text{max}}$ ($p=0.006$) and anaerobic capacity ($p=0.01$). **Conclusions:** 4 weeks of ALT training increases blood O_2 content due to an increase in RCV which increases $VO_{2\text{max}}$ and running performance over 5000m, and which may be improved most by living at altitude and training near sea level.

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THE RIGHT VENTRICLE DURING EXERCISE AT HIGH ALTITUDE

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High altitude results in a predictable decrease in maximal exercise capacity. However, the mechanism for this decrease is not clear. Left ventricular (LV) filling is reduced and maximal stroke volume (SV) is low compared to sea level (SL) exercise, despite maintenance of LV contractile function. We hypothesized that the right ventricle (RV) may be unable to increase its ejection in the face of pulmonary hypertension induced by hypoxia and might therefore impair LV filling and exercise performance. To test this hypothesis, we studied 11 patients (age 14.5±5.3) without a RV pumping chamber, ie. after the Fontan operation in whom the systemic venous return passes directly into the pulmonary circulation. Each subject was tested at SL and at simulated altitude of 3048m (522 torr) (AL). Cardiac output (Q , C_6H_6 rebreathing), oxygen uptake (VO_2 , Douglas bag), and HR (ECG) were measured. Stroke volume (SV) and $a \cdot v_0$ difference were calculated. At the same absolute submaximal workrate (21±10 watts), HR was higher at AL (106±8 vs. 119±20*), though VO_2 and Q were not different. For peak exercise (χ^2 sd; * $p<0.05$ compared to SL):

Peak	VO_2	$a \cdot v_0$	diff	HR	SV
Data	(ml/kg/m)	(l/min)	(ml/dl)	(bpm)	(ml)
SL	23.5±5.3	8.8±1.9	12.6±3.0	162±13	54.1±10.9
AL	17.8±4.0*	7.3±2.9*	12.0±2.4	162±8	45.4±17.3*

Although SV fell at peak exercise, the reduction in $VO_{2\text{max}}$ at 3048m (24%) in these patients was close to the reduction expected in normal individuals (15%). Conclusion: Normal right ventricular pump function is not necessary for exercise performance at altitude.

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INCREASED CAPILLARY PERMEABILITY IN ACUTE MOUNTAIN SICKNESS (AMS)

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Hypoxia induced increase of capillary permeability has been proposed as a mechanism for AMS. A unique multi-step computer controlled limb plethysmography technique was used to investigate capillary filtration coefficient (CFC) in seven subjects (2F, age 25-54 years) who were studied at baseline altitude of 1500m and after helicopter transfer to 4500m. Occlusion pressure applied in 10mmHg steps via a thigh cuff caused an initial limb volume increase due to venous engorgement and thereafter fluid filtration from capillary to interstitium. Volume changes were measured by mercury in siliastic strain gauge. AMS symptoms were assessed by Lake Louise and Birmingham altitude sickness scoring systems. CFC, gradient of fluid flux and cuff pressure plot, increased from 3.3 (21-4.5) median and range, at 1500m to 4.6 (2.9-8.3) after 24 hours at 4500m. Units $\times 10^{-3}$ /min/100g tissue/mmHg, $p=0.02$. Change in CFC shows a positive but non significant correlation with AMS symptoms. The results support the hypothesis of systemic capillary leakage as part of the syndrome of acute mountain sickness.

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EFFECTS OF BAROMETRIC PRESSURE ON VENTILATORY RESPONSE TO REDUCED P_O_2

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In 1983, Tucker *et al.* (Resp. Physiol. 54:363) reported a greater ventilatory response to normobaric hypoxia (NH) than to simulated altitude (SA) within two hours of the same reduction in P_O_2 and S_O_2 . We noted parallel trends during the tenth hour under similar altitude and hypoxic conditions (FASEB J. 8:A553, 1994). Inconsistencies in calculated anatomical deadspace in both studies led us to continue investigation of this apparent attenuation of ventilation by reduced barometric pressure. We serially exposed subjects at 30 min intervals to control, normobaric hypoxia ($P_B = 640$ mm Hg, $F_O_2 = 0.14$, $P_I O_2 = 82$), hypobaric normoxia ($P_B = 435$ mm Hg, $F_O_2 = 0.31$, $P_I O_2 = 122$) and SA ($P_B = 435$ mm Hg, $F_O_2 \approx 0.21$, $P_I O_2 = 82$). Preliminary results from three subjects resulted in minute ventilation, end-tidal PCO_2 and S_O_2 responses that were not consistent with accepted steady-state assumptions of gas exchange. We also noted that heart rate and sensations of hypoxia were greater during SA than NH. This apparent dependence on barometric pressure of the ventilatory response to reduced P_O_2 deserves further investigation.

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FLUID AND ELECTROLYTE SHIFTS DURING EXERCISE WITH A SMALL MUSCLE GROUP UNDER ACUTE HYPOXIA

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Exercise with a large muscle group under acute hypoxia (HO) leads to an enhanced increase of $[K^+]$ compared to normoxia (NO). It is not known whether this is a direct effect of the reduced P_O_2 on the working muscle. Methods: 5 male subjects performed incremental handgrip exercise under acute HO (12.5% O_2), NO , and hyperoxia (50% O_2 , HY). Skin blood flow was reduced by cooling. Blood was drawn from an arterialized hand vein and a cubital vein. P_O_2 , $[K^+]$ and $[Na^+]$ in plasma and blood flow (plethysmography) were measured. Results: $[K^+]$ in arterIALIZED blood remained almost constant during exercise. The level was slightly but not significantly different under the various conditions. Venous $[K^+]$ increased to 5.8±0.5 (NO), 5.9±0.6 (HO) and 5.5 mmol/l (HY , not significantly different). Under all conditions $[Na^+]$ increased linearly by about 1 mmol/l in arterIALIZED blood and about 7 mmol/l in venous blood, respectively. The increase in blood flow did not differ significantly during HO , NO , and HY . Conclusion: The enhanced increase of $[K^+]$ during cycle exercise under hypoxia seems not to be an effect of the reduced P_O_2 on the muscle, per se.

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INHALED NITRIC OXIDE INHIBITS PULMONARY VASCULAR IMPEDANCE RESPONSES TO HYPOXIA IN DOGS AND MINIPIGS

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The pig has been reported to present with a stronger hypoxic pulmonary vasoconstriction than in many other species, including man. We investigated pulmonary vascular impedance (PVZ) spectra and pulmonary artery pressure (P_{pa}) minus pulmonary artery occluded pressure (P_{pao}) versus pulmonary blood flow (Q) in anaesthetised and ventilated minipigs and 9 weight-matched dogs. Animals were sequentially exposed to hyperoxia ($F_O_2 0.4$), hypoxia ($F_O_2 0.12$) without and with nitric-oxide (NO) inhalation (150 ppm). Flow matched PVZ data (mean±SEM) are shown in the table. $Z_0 = 0$ Hz impedance (Z), $Z_1 =$ first harmonic Z , $Z_c =$ characteristic Z , $Z_1 ph =$ first harmonic phase angle, f , $\#$, $*$ at least $p < 0.05$ between $F_O_2 0.4$ and 0.12, $F_O_2 0.4$ and $F_O_2 0.4\&NO$, dog (d) and minipig (m) respectively]

F_O_2	0.4	0.12	0.4&NO	0.12&NO
Z_0 d	433±33	534±66 f	422±33	438±38
	779±51 *	1339±123 *	728±61 *	846±70 *
Z_1 d	107±12	119±15	102±16	86±12
	147±13 *	246±33 f*	147±18	150±19 *
Z_c d	100±13	102±12	112±20	94±13
	117±11	158±23 f*	127±15	121±12 *
$Z_1 ph$ d	-0.43±0.1	-0.46±0.1	-0.26±0.1 #	-0.24±0.1
	-0.85±0.1 *	-0.85±0.1 *	-0.74±0.1 *	-0.88±0.1 *

In hyperoxia, compared to dogs at the same Q , minipigs had a higher P_{pa} (26±1 mmHg versus 16±1 mmHg; $p < 0.01$). Hypoxia increased P_{pa} (P_{pa} - P_{pao}) at all level of Q by an average of 13 mmHg in minipigs and 2 mmHg in dogs. Inhaled NO inhibited hypoxia induced (P_{pao} - P_{pa})/ Q changes in both animal species. We conclude 1st that the minipig is an animal model of elevated pulmonary vascular resistance and impedance, and 2nd that hypoxia induced alterations in PVZ spectrum are due to small arteries resistance changes.

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ACETAZOLAMIDE AND ALMITRINE IN ACUTE MOUNTAIN SICKNESS (AMS) TREATMENT

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At the "cappanna Regina Margherita" (4559m) during July and August 1993-94 55 volunteers were, prospectively randomised to receive acetazolamide (Ac) (250mg; n=14), dexamethasone (Dx) (4mg; n=14), almitrine (Al) (100mg; n=14) or placebo (Pl) (n=13) in a double-blind manner. Purpose of the study was to compare AMS treatment efficacy of two different respiratory stimulants as an alternative to Dx. After informed consent was obtained climbers with a clinical AMS score of ≥ 4 (BMI 1990, 301:853-5) were entered in the study. AMS severity (Lake Louise score [LLS]; AMS-R subscore) and blood gas analyses were obtained before (b) and 10-12 hours after (a) a treatment start. After the initial oral administration medication was repeated 8 hours later. Results (mean \pm SEM) are shown in the table.

	Ac	Dx	Al	Pl	p value
LLS	b 9 \pm 1	8 \pm 1	8 \pm 1	8 \pm 1	ns
a	7 \pm 1 (*)	5 \pm 1 **	8 \pm 1	8 \pm 1	<0.05
AMS-R	b 0.8 \pm 0.1	0.8 \pm 0.1	1.1 \pm 0.2	0.8 \pm 0.1	ns
a	0.6 \pm 0.1 *	0.4 \pm 0.1 *	0.9 \pm 0.1	0.8 \pm 0.1	<0.05
PaO ₂	b 35 \pm 1	36 \pm 1	35 \pm 1	36 \pm 1	ns
a	41 \pm 1 **	43 \pm 1 **	39 \pm 1 **	38 \pm 2	ns
(A-a)PO ₂	b 14 \pm 1	14 \pm 2	12 \pm 1	11 \pm 1	ns
a	10 \pm 1 **	8 \pm 1 **	13 \pm 1	11 \pm 2	<0.05

{(*) p = 0.05, * p < 0.05, ** p < 0.01 compared to before treatment)
Blood pH decreased from 7.48 \pm 0.02 to 7.43 \pm 0.03 (p<0.01) in Ac treated climbers whereas remained unchanged in the other groups.

This study shows that acetazolamide and dexamethasone significantly improve gas exchange in AMS subjects, but that the latter relieves AMS associated symptoms more efficiently. Despite an increase of

PaO₂ almitrine failed to relieve AMS symptoms.

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SPIROMETRY AND CHRONIC HYPOXIA

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Chronic hypoxia causes a decrease in expiratory flow rate in adults with chronic obstructive pulmonary disease which is reversible with oxygen (Libby et al, 1981). We used altitude as a model to study the effect of chronic hypoxia on spirometric measurements using a pocket turbine spirometer. We administered oxygen for 5 minutes to 47 subjects (age range 19-55 years) on the British Mount Everest Medical Expedition 1994 at Everest Base Camp (5300m) using an open circuit system at 1L/min via a face mask. Mean oxygen saturation rose from 80.7% before oxygen to 93.5% after 5 minutes of oxygen. There was no significant effect on FEV1 or FVC. PEF fell by 2.3% (p<0.01). Thirty-four non-asthmatic subjects were given salbutamol which produced no significant change in FEV1, FVC or PEF. Four asthmatics had a mean rise in FEV1 of 10% and PEF increased by 5%.

We have not been able to demonstrate any improvement in PEF, FEV1 or FVC after administration of oxygen or salbutamol at 5300m except in asthmatics who responded to bronchodilator.

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POSSIBLE PRESENCE OF HYPOXIC VENTILATORY DEPRESSION WHILE BREATHING AMBIENT AIR AT SEA LEVEL IN HUMANS

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Ventilatory response to sustained hypoxia is biphasic and the secondary decline after an initial brisk response is named hypoxic ventilatory depression (HVD). Several studies have suggested the magnitude of HVD is related with the amount of the centrally mediated peripheral chemoreceptor drives. To test the significance of the peripheral chemosensitivity, 14 healthy adults were examined their hypoxic ventilatory response (HVR) following 10 min hypoxic and control normoxic exposure (defined O_2 and O_2 runs, respectively). During HVR test, end-tidal PCO_2 was tried to keep constant at the level of room air breathing in both O_2 (39.1 \pm 4.2 mmHg) and O_2 (3.2 \pm 4.8 mmHg) runs. Seven subjects consistently showed higher HVR (Ve/SaO_2) in O_2 runs ($-0.49\pm 0.21 \text{ l/min}/\text{SaO}_2$) than O_2 runs ($0.18\pm 0.12 \text{ l/min}/\text{SaO}_2$) and defined as the positive responders. The remaining 7 subjects (defined the negative responders) revealed inconsistent response to O_2 exposure. It was also noted that the magnitude of HVR in the positive responders while breathing ambient air ($0.18\pm 0.12 \text{ l/min}/\text{SaO}_2$) was lower than the negative responders ($0.49\pm 0.28 \text{ l/min}/\text{SaO}_2$) and elevated significantly larger than the latter following hyperoxic exposure. We conclude that the positive responders exhibit HVD during ambient air breathing at the sea level.

Part of this work was supported by the Ministry of Education of Japan.

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PRESERVED VAGAL TONE AND FRACTAL PROPERTY IN HR VARIANCE IN SHERPA HIGHLANDERS.

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Sympatho-vagal balance and fractal quality of HR variance were investigated by analyzing R-R intervals of ECG in 10 healthy male Sherpa highlanders living at around 3500m in Khumbu Nepal and 8 lowlander trekkers in early acclimatizing phase. Electric signals from the chest lead for longer than 10 minutes at resting state were directly sampled by 100Hz at altitude of 4200m. Power spectral analysis of the R-R interval was done by both FFT and MEM methods to calculate low frequency(LF) and high frequency(HF) component. Fractal property were evaluated by the fractal dimensions which were calculated by the relative dispersion analysis method and Hurst's rescaling analysis method. Though no significant difference was found in R-R intervals between two groups, Sherpa group showed significantly higher SpO_2 , higher HF/(LF+HF) and lower LF/HF ratio in power spectral analysis, which implied that they preserved vagal activity in contrast to lowlanders' sympathetic dominance to vagal tone at altitude exposure. Sherpa group kept the fractal property of R-R variances and the dimensions did not differ from that of trekkers from lowland nor of lowlanders at sea level. It is suggested that preserved vagal and less sympathetic activity was an evidence of well acclimatization at altitude in Sherpa highlanders.

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HYPOXIC VENTILATORY RESPONSE AND PULMONARY GAS EXCHANGE DURING EXPOSURE TO HIGH ALTITUDE IN SUBJECTS SUSCEPTIBLE TO HIGH-ALTITUDE PULMONARY EDEMA (HAPE)

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The relative influence of alveolar hypovenitalation and impaired pulmonary gas exchange on exaggerated hypoxemia which occurs in subjects susceptible to HAPE (HAPE-S) during early period of high-altitude exposure was examined. Nine HAPE-S aged 29 ± 8 yrs and six control subjects were exposed to a simulated altitude of 3,200m ($P_{\text{a}} = 515$ Torr). Before and 20 min after arrival, simultaneous sampling of expired gas and arterial blood was performed for the calculation of alveolar-arterial O_2 difference ($A-aO_2$). Results were (mean \pm SD):

	Baseline(610m)		3,200m
PaO ₂ HAPE-S	91.1	± 4.1	53.2 $\pm 2.2^{*}$
Control	90.2	± 3.0	57.0 $\pm 2.9^{*}$
PaO ₂ HAPE-S	88.8	± 5.3	47.4 $\pm 4.7^{*}$
Control	85.7	± 4.9	52.6 $\pm 2.1^{*}$
A-aO ₂ HAPE-S	2.4	± 1.2	5.8 $\pm 3.7^{*}$
Control	4.5	± 2.8	4.3 ± 2.0

*p<0.01 vs baseline, #p<0.05 vs control. The relative hypovenitalation observed in HAPE-S at 3,200m was related to the sea-level hypoxic ventilatory response. HAPE-S also exhibited a significant increase in A-aO₂ at 3,200m compared with baseline, and two of them had A-aO₂ of more than 10 Torr. These results suggest that both hypovenitalation and a widened A-aO₂ may contribute to the exaggerated hypoxemia in HAPE-S.

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INHERITANCE OF HYPOXIC EXERCISE TOLERANCE DIFFERENCES IN MICE

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The aim of this study was to characterize the inheritance of hypoxic exercise tolerance (t_{et} , time to a behavioral endpoint) differences in mice exposed for 3 weeks to 380 Torr. Four lines of evidence for predominant involvement of 2 unlinked autosomal genes are presented: (1) application of a maximum likelihood procedure to t_{et} distributions of 2 parental inbred strains, their F_1 hybrid, and the backcross (BC) generations revealed a "best fit" to a 2 major locus inheritance model; (2) repeated cycles of selecting as the progenitor of a new BC generation the male with the highest value of t_{et} in the previous BC generation, and breeding him to females of one of the original parental strains produced BC distributions suggestive of 2 locus inheritance--2 distinct t_{et} phenotypic classes that were, moreover, stable as shown by close similarity of quantile-quantile data plots; (3) results of breeding tests involving recombinant inbred (RI) lines were consistent with the 2-locus mode and permitted assignment of putative genotypes to each RI line and to the BC phenotypes; and (4) breeding mice from each BC phenotype to each other and to the other phenotypes and to the parent strain suggested a 2 gene mode. A positional cloning strategy using DNA simple sequence repeats to map the genes is underway.

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METABOLIC RATE AND CELLULAR PROTECTION BY GAL-XI DURING ATP DEPLETION IN WIF-B CELLS

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Objectives: 1) Establish a convenient cellular model to investigate liver hypoxia; 2) correlate metabolic rate with cell viability during mitochondrial uncoupling and 3) assess cytoprotection of GAL-XI by metabolic rate and intracellular enzyme leakage.

Methods: 1) WIF-B cells - a polarized hepatoma-derived hybrid were employed throughout. Cells were grown in a modified Hams F-12 medium on a microporous polycarbonate transwell and studied at 5 days following seeding. 2) Metabolic rate was assessed by pH-sensitive silicon sensor, a light addressable potentiometric sensor, interfaced to a 3 μ L flow chamber, with continuous measurement of the extracellular acidification rate. Cytosol contained balanced salt solution (BSS), either 10 mM glucose or 10 mM GAL-XI, \pm 100 μ M DNP. 4) Cellular efflux was collected every 10 min, and analyzed enzymatically for lactate dehydrogenase (LDH) content expressed as % released relative to total cell content.

	BSS, GLUCOSE		BSS, GAL-XI	
	DNP (μ M)			
METABOLIC RATE*	80	40	200	60
LDH RELEASE†	0	80	0	20

*Expressed as % relative [μ vol x sec $^{-1}$], to basal activity obtained in BSS.

†Expressed as % release relative to total cellular LDH content.

Summary: 1) WIF-B metabolic rate is diminished by 50% when DNP is added to a perfusate of 10 mM glucose- this is accompanied by significant cell injury evidenced by 80% LDH release. 2) GAL-XI enhances metabolic rate by ~ 2.5 fold in comparison to glucose. 3) Addition of DNP to GAL-XI perfusate decreased metabolic rate to 60%, however, only 20% LDH release was recorded suggestive of cellular protection by GAL-XI. Studies are ongoing to define the mechanism of GAL-XI mediated cytoprotection in this model of hepatic anoxia.

¹Molecular Devices Corporation; Special thank you to Dr. A. Hubbard for WIF-B cells. Supported in part by the Advanced Research Projects Administration [ARPA].

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LONG TERM FACILITATION OF VENTILATION IN DUCKS; LEARNING TO BREATHE IN HYPOXIA

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White Pekin Ducks were exposed to ten, three minute episodes of hypoxia (9% O_2) interspersed with five minute episodes where they returned to breathing room air. The series was followed by a one hour recovery period during which the birds breathed air. Birds were used for this study because of the ease with which arterial blood could be maintained isocapnic throughout the entire experiment by unidirectional perfusion of the respiratory system. This allowed us to test the hypothesis that long-term facilitation occurs in response to hypoxic exposure. During each hypoxic exposure, ventilation showed an initial increase (acute response) followed by a further slow increase in both tidal volume and breathing frequency (short term potentiation). The magnitude of the increase in ventilation which occurred during each hypoxic exposure increased from the first through the last hypoxic exposure (progressive augmentation). Finally the level of ventilation observed after each hypoxic episode slowly increased and by the tenth hypoxic episode, the level of ventilation exhibited by the birds while breathing air had increased by 56%, primarily due to an increase in tidal volume. It took over an hour for the ventilation to return to pre-hypoxic levels. Thus ducks also exhibit long-term facilitation of ventilation on exposure to hypoxia. These data reveal a number of different types of neuromodulation which enhance the ventilatory response to hypoxia in response to previous respiratory challenge.

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ABSTRACTS

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COMPARISON OF RESPIRATORY AND CIRCULATORY INTERACTION DURING PROGRESSIVE ISOCAPNIC HYPOXIA AND ACUTE HYPOBARIC HYPOXIA.

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This study was designed to investigate the difference in respiratory and circulatory interaction between progressive isocapnic hypoxia (PIH) and acute hypobaric hypoxia (AHH). Six subjects (5 males and 1 female) were exposed to PIH followed by AHH. V_t , f and \dot{V}_E were continuously measured by a hot-wire flowmeter, end-tidal PO_2 and PCO_2 by a rapid response gas analyzer and SpO_2 by a pulse oximeter. SV, HR and CO were also continuously recorded by an impedance cardiography using a computer-based, on-line system. BP was measured by a non-invasive finger-cuff method. $\Delta HR/\Delta SpO_2$ was greater during PIH than during AHH. Ventilatory response to hypoxia, which was definitely existed during PIH, turned blunted during AHH with $PaCO_2$ remained constant in both trials. $\Delta HR/\Delta SpO_2$ was positively correlated with $\Delta \dot{V}_E/\Delta SpO_2$. SV was slightly decreased during PIH, but not in AHH. SV was negatively correlated with HR. No significant change in CO was observed during PIH, but an increasing tendency was seen during AHH. There was no consistent tendency in BP change during both PIH and AHH. These results suggest that the interaction between respiratory and circulatory systems exhibited in normobaric hypoxia was modified by hypobaric hypoxia.

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ARTERIAL OXYGEN TENSION AT MODERATE ALTITUDE: A MULTIVARIATE MODEL FOR CHRONIC OBSTRUCTIVE LUNG DISEASE

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Previous studies have shown that individuals with chronic obstructive lung disease may develop severe hypoxemia upon ascent to moderate altitude. It has also been demonstrated that the degree of hypoxemia in such individuals correlates reasonably well with baseline values of PaO_2 and FEV_1 measured at ground altitude¹⁻⁵. The data from 72 subjects in five published studies¹⁻⁵ were pooled and analyzed by multiple linear regression. The following multivariate model was developed for predicting arterial oxygen tension at moderate altitude ($PaO_2[ALT]$) in subjects with chronic obstructive lung disease:

$$PaO_2[ALT] = 0.19 (FEV_1 * PaO_2[GN]) - 11.51 [\ln (MA - GA)] + 123.17$$

where $PaO_2[GN]$ is arterial oxygen tension at ground altitude, MA is the moderate altitude to which the subject ascends and GA is the ground altitude at which baseline measurements were made. This model has excellent predictive power ($r^2 = 0.99$) at a high level of statistical significance ($p = 0.01$). It demonstrates that baseline FEV_1 and PaO_2 measured at ground altitude prior to ascent can be used to accurately estimate the arterial oxygen tension at moderate altitudes in individuals with chronic obstructive lung disease.

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HEMOGLOBIN AFFINITY AND STRUCTURE IN HIGH-ALTITUDE AND SEA-LEVEL CARNIVORES FROM PERU.

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Numerous studies of mammalian and avian species native to hypoxic environments demonstrate the adaptive significance of high oxygen affinity hemoglobins. We compared here the hemoglobin affinity (P50) and hemoglobin (Hb) structure of high altitude carnivores with populations of the same species or the same genus living at sea level. The P50 was measured in cat (*Felis jacchetta*), puma (*Felis concolor*) and fox (*Dusicyon culpaeus* and *D. sechurae*) by a mixing technique. The heme and globins were fractionated by HPLC with a linear gradient. P50 differed significantly in the animals occupying the two niches and pumas and foxes showed structural differences in their hemoglobins. These findings show that the P50 is not only different (lower) in the high altitude genetically adapted species but also it can differentiate groups of the same species living at sea level from those occupying the high altitude niche. Phylogenetic aspects of Hb evolution under the effect of the hypoxic selecting force can also be inferred from the presented data.

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ROLE OF VASOCONSTRICTOR SYSTEMS IN THE SYSTEMIC HYPERTENSION OF RATS ACCLIMATIZED TO SIMULATED ALTITUDE.

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Rats acclimatized to PB 370 Torr (A rats) show higher mean systemic arterial blood pressure (MABP) than non-acclimatized (NA) litter mates (J. Appl. Physiol. 77:1341-1348, 1994). To determine the role of the major vasoconstrictor systems in this phenomenon, the effect of blockade of α -adrenergic (AD), angiotensin II (AII) and arginine-vasopressin (AVP) receptors on MABP was studied in conscious, resting A and NA rats breathing 10% O_2 . Control MABP was higher in A rats (123 \pm 2 vs 92 \pm 2 mmHg, p<0.05). Blockade of AII or of AVP receptors influenced MABP in A and NA only after AD receptor blockade, but did not affect baseline MABP prior to AD block. When both AVP and AII receptors were blocked, AD receptor blockade lowered MABP by 62 \pm 2 mmHg in A rats and by 41 \pm 2 mmHg in NA (p<0.05). The time course of the MABP decrease was different: in A, it took over 20 min, with 60% occurring in the 1st min; in NA it was completed within 2 min. The smooth muscle relaxant sodium nitroprusside (SNP) did not further modify MABP in either group, which reached 61 \pm 1 mmHg in A and 51 \pm 2 mmHg in NA rats (p<0.05). In conclusion: 1. Neither AII nor AVP contribute to baseline MABP regulation in either A or NA, or to the hypertension of A; 2. The larger decrease in MABP following AD blockade suggests a higher α -adrenergic tone in A rats; 3. The lack of effect of SNP after all 3 systems were blocked suggests that no other system influences MABP in hypoxia; and 4. The higher MABP after SNP may reflect the higher hematocrit and/or vascular remodeling in A rats. Supported by NIH grant HL39443.

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MODULATION OF HYPOXIC PULMONARY VASOCONSTRICITION IN CONSCIOUS DOGS.

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We tested the hypothesis that the magnitude of hypoxic pulmonary vasoconstriction (HPV) is modulated by humoral and local mechanisms. Left pulmonary vascular pressure-flow (LPQ) plots were generated in chronically-instrumented conscious dogs by continuously measuring the pulmonary vascular pressure gradient (PAP-LAP) and left pulmonary blood flow (LQ) during gradual inflation of a right pulmonary artery occluder. Studies were performed on separate days in the intact condition and then after either: AVP, V₁ receptor block ($d(\text{CH}_2)_5$ AVP), angiotensin II receptor block (saralasin), cyclooxygenase inhibition (indomethacin), or ATP-sensitive K⁺ channel (K^{ATP}) block (glybenclamide). LPQ plots were obtained during normoxia (PaO₂ ~ 95 torr) and hypoxia (PaO₂ ~ 50 torr). Administration of the blockers had no significant effect on the baseline LPQ relationship. Increases in PAP-LAP (mmHg) from normoxia to hypoxia at LQ = 100 ml/min/kg are summarized below. HPV (*p<0.01) was observed in all groups. AVP, V₁ receptor block and angiotensin II receptor block did not alter the magnitude of HPV. In contrast, both cyclooxygenase inhibition and K^{ATP} channel block potentiated HPV (*p < 0.01). Thus, in the conscious state endogenous AVP and angiotensin II do not modulate HPV, whereas cyclooxygenase metabolites and K^{ATP} channel activation attenuate HPV.

	d(CH ₂) ₅ AVP	Saralasin	Indomethacin	Glybenclamide
Intact	11 ± 2*	11 ± 2*	10 ± 1*	11 ± 1*
Blockers	13 ± 2*	11 ± 2*	15 ± 2*	14 ± 1†

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STAGED VS RAPID ASCENT TO INTERMEDIATE AND HIGH TERRESTRIAL ELEVATIONS: EFFECTS ON SUBMAXIMAL EXERCISE PERFORMANCE.

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Staging at moderate elevations is believed to improve submaximal exercise performance upon arrival at higher altitudes, however, data supporting this notion are sparse. We studied the effect of three days of staging at moderate altitude (~1,830 m) on the exercise performance and physiological strain of lowlanders at intermediate, (1,274 m) and high (H:4,300 m) terrestrial elevations. Nine male subjects (Ss) performed a lift and carry task (~26 kg carried 10 m, 4x/min @ $\dot{V}O_2$ -1.7 l·min⁻¹) for 10 min. Exercise duration (ED), $\dot{V}E$, $\dot{V}O_2$, pulse oximetry (SaO₂), heart rate (HR), and rated perceived exertion (RPE) were measured following rapid (R:<12 h) and staged (S:>72 h) ascents. At I, ED was unaltered (8/8 Ss completing) following either R or S ascents. However, after S ascent, SaO₂ was higher (MEAN±SD: 89±2 vs 85±3%) and $\dot{V}O_2$ pulse larger (11.3±1.1 vs 10.3±1.2 ml·beat⁻¹). At H, ED was reduced following R ascent in 3 Ss (5±3 min) but following S ascent, all 8 Ss completed the target ED. However, S ascent had no measurable effect on physiological strain at H. These results suggest that although rapid ascent to I did not impair exercise performance at I altitude, subsequent staging did decrease physiological strain. Whereas at H, exercise performance was improved by staging, although physiological strain was not apparently abated at this high altitude by staging.

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THE VOLUNTARY BREATH HOLDING TIME(VBHT) AT HIGH ALTITUDE.

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The shortening of Voluntary Breath Holding Time (VBHT) at altitude was studied with Kyoto University Pamirs Scientific Expedition 1993(KUPSE'93), Kyoto University Medical Research Expedition to Kixabangma 1990(KUNREX'90) and Japanese Mt. Everest Expedition 1970(JMEE'70).

Because of wide range of individual variation, the data were summed and discussed on VBHT RATE(=VBHT at altitude/VBHT at sea level×100).

The results revealed that the shortening of VBHT is not a phenomenon at high altitude only but it starts from sea level and the grade of slope of VBHT-Barometric Pressure curve reduced gradually with lowering of pressure(increasing of altitude). During sojourn at same altitude, VBHT recovered a little, then shortened again. This tendency was observed commonly through these 3 Expeditions. The second shortening of VBHT may be due to physical fatigue of the members. In 1949, Rahn and Otis published that shortening of VBHT reflected the acclimatization and grade of shortening is the good indicator of acclimatization, this theory can not explain the recovery of shortening during sojourn. Fatigue might rather be an important factor of shortening besides acclimatization after several days sojourn.

As the conclusion, the shortening of VBHT may not reflect only high altitude acclimatization but high altitude deterioration. Measuring VBHT may be a good standard to control physical condition at high altitude.

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ALBUMIN DISTRIBUTION VOLUME AND PLASMA VOLUME DURING ACCLIMATIZATION TO HYPOXIA

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Acute mountain sickness (AMS) is associated with hemoconcentration and generalized edema. The mechanism of the altered distribution of fluid is unknown. We have studied the volume of distribution of albumin relative to the volume of plasma in eight volunteers during acclimatization to high altitude (4,350 m). The volume of distribution of albumin was determined with Evans' blue. Plasma volume was determined as (1-Hct) * BV, where Hct was the venous hematocrit, and BV was the blood volume measured by a carbon-monoxide rebreathing method. After one day in hypoxia, the albumin distribution volume was unaltered 3.5 L. Plasma volume by the carbonmonoxide method decreased from 3.4 to 3.0 L (P<0.05). The ratio between the volume of distribution of albumin and the plasma volume increased from 1.04 to 1.17 (P<0.05), indicating that the compartment of albumin below the endothelium was increased at the expense of plasma volume. We suggest that a re-distribution of albumin may be important in AMS, but further studies are needed.

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TREATMENT OF ACUTE MOUNTAIN SICKNESS IN HIMALAYAN TREKKERS: A PRELIMINARY PROSPECTIVE RANDOMIZED TRIAL OF HYPERBARIA VERSUS DEXAMETHASONE

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Dexamethasone and hyperbaria are effective treatment in Acute Mountain Sickness(AMS). We hypothesized the treatments were equally effective. Eleven trekkers(7 M/4 F aged 21-61y) with Lake Louise AMS scores >4 were randomized at 4250m or 4930m. Six trekkers underwent hyperbaria(Hb) for two hours and five were given dexamethasone(D) 4mg po at 0 and 6h. AMS scores at entry were similar (Hb=5.83 ± 1.47 and D=7.40±1.95). At 2h the Hb group was improved(AMS score 0.67 ± 0.52) versus the D group (AMS score 3.20±1.64; p<0.01). At 6 and 12h there were no differences between the Hb (AMS 1.67±1.97 and 0.83±1.17) and D groups (AMS 2.00 ± 1.00 and 1.00±1.73;p=ns). At 24h scores were lower in the D group (D=0.20±0.45 and Hb=2.00 ± 1.67; p=0.03). The repeated measures analysis of variance indicated a significant decreasing trend over time but that the trend differed between the two groups(p<0.008). We conclude, in this small study, that while hyperbaria offers significant early symptom relief, dexamethasone is equivalent at 6h and 12h and significantly better at 24h in the treatment of AMS in Himalayan trekkers.

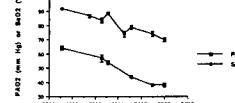
Supported by University of Vermont, Himalayan Rescue Association and Denali Medical Research Foundation

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RESPIRATORY FUNCTION AT EXTREME ALTITUDE: results from the British 40th anniversary Everest expedition.

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The success of the 1953 Everest expedition was due in large part to the physiological studies of Griffith Pugh. Since then there have been many physiological studies including the AMBREE 1981 study where PAO₂ was measured up to the summit of Everest (8840m) and Operation Everest II (OEII) where PAO₂ and SaO₂ were measured during a simulated climb to the same altitude. To our knowledge there are no field studies where there has been a comparison of alveolar PO₂ and arterial oxygen saturation at altitudes as high as 8000 m and there is controversy about the effect of extreme altitude on gas exchange in acclimatized subjects. One of the main aims of the British 40th Anniversary expedition was to determine spirometry and the relationship between PAO₂ and SaO₂ in 9 elite climbers (aged 32-53; BM: 1F attempting the summit. We measured SaO₂ using a portable pulse oximeter (prepac Ltd Lancs, UK), PAO₂ using an oxygen fuel cell (Teldyne, Keighley, UK) attached to a digital volt meter and FEV₁/FVC using a portable spirorometer (Escort, Vialograph, UK). Spirometry was performed at altitudes up to Camp 2 at 6550 m. Measurements of SaO₂ and PAO₂ were made at rest breathing air up to and including the South Col (8000 m). None of the climbers developed overt high altitude pulmonary oedema and none demonstrated any change in FEV₁ or FVC. The changes in PAO₂ and SaO₂ with altitude are shown below.



The graph shows a small decline in PAO₂ and SaO₂ at these levels of 38 ± SD 1.3 mm Hg and 70 ± 2.5% respectively. It is interesting that the PAO₂ did not fall below 36 mm Hg which is similar to the result found in the AMBREE study (PAO₂ = 37 mm Hg at 8400m) but higher than that found in OEII (31 mm Hg at 8000m) suggesting that this PAO₂ is defended by hyperventilation in acclimatized subjects. In our subjects, the SaO₂ fell even when PAO₂ was stable suggesting V/Q mismatching possibly due to mild high altitude pulmonary oedema.

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CHANGES IN SPIROMETRY AT ALTITUDE

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Peak expiratory flow (PEF) rises with increasing altitude because of the decreased air density but devices for measuring PEF which are themselves affected by air density under-read. We collected spirometric data from 52 members (aged 19-55 years) of the 1994 British Mount Everest Medical Expedition at sea level (1012.1-1015.5mB) and at Everest Base Camp (5300m, 530-547mB) with a pocket turbine spirometer (Microspirometer, Micro Medical, UK), which is not affected by air density, and compared its performance with the density dependent Mini-Wright Peak Flow Meter. Mean oxygen saturation at 5300m was 80.7% (0.75) and 97.6% (0.33) at sea level. Using the Microspirometer, there was no significant change in FEV₁ (p>0.1); FVC fell by an average of 5.3% (0.94; p<0.001), and PEF rose by 23.5% (2.09; p<0.001). PEF, measured with the MiniWright Peak Flow Meter, fell by 7% (1.24; p<0.001). We did not show a lower PEF in individuals with acute mountain sickness (AMS). The fall in FVC has been previously attributed to interstitial oedema but we found no change in FVC after 7 or more days of acclimatisation compared with the first three days at Everest Base Camp. This data confirms the rise in true peak flow at 5300m and shows that the MiniWright Peak flow meter under-reads by 30% at 5300m. Supported by Micro Medical UK, SmithKline Beecham, Zeneca.

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HYPOXIC HYPOTENSION(HH) IN RATS: CIRCULATING AND TISSUE LEVELS OF ENDOTHELIN(ET).

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Breathing and gas exchange monitored (pneumoresistors, fast pressure transducer, mass-spectrometer, analog-to-digital converter and computer). ET by RI assay in jugular and carotid plasma and homogenates (brain, liver lungs). Blood gases, pH, Hbgl. and lactic acid, (right atrial blood). Anesthetized rats, instrumented after tracheotomy. Controls(12), room air 30min. HH(6% oxygen 30min.) in 16. HH reduced blood oxygen tension (23.40±0.37torr) but produced no changes in ET in plasma or tissues. Correlations of ET and body mass - ET_{lung} r=-0.839±0.027, ET_{liver} r=-0.980±0.0003 and in controls, duration of inspiration(TI)-ET_{lung} r=-0.929±0.014, ET_{brain} r=-0.931±0.014 and tidal volume(VT) ET_{lung} r=-0.907±0.019, ET_{brain} r=-0.853±0.025. Increased ET_{lung} produced increased lung elastance in controls and HH. Increased ET_{lung} and ET_{brain} produced significant increased airway resistance.

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HYPOXIC HYPOXIA(HH) IN RATS: CIRCULATING AND TISSUE LEVELS OF ENDOTHELIN(ET): EFFECTS OF EXOGENOUS PHOPHOLIPIDS ("LIPIN", LPN).

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Breathing and gas exchange monitored (pneumoresistors, fast pressure transducer, mass-spectrometer, analog-to-digital converter and computer). ET by RI assay in jugular and carotid plasma and homogenates (brain, liver lungs). Blood gases, pH, Hbgl. and lactic acid, (right atrial blood). Anesthetized rats, instrumented after tracheotomy. Controls(C's 12), room air 30min. HH(6% oxygen 30min.) 16; iv-LPN followed by HH(16). LPN no effect on ET. LPN increased O₂ consumption, decreased lactate and lipid peroxides and increased pH during HH. Lung diffusion capacity increased, elastances decreased. Inspired volume acceleration, C=153±28, HH=277±40, HH+LPN=163±22±2 HH abolished correlation between duration of inspiration(TI) and ET_{lung}, this was restored by LPN.

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CARDIAC OUTPUT, PULMONARY DIFFUSION AND DEAD SPACE VENTILATION IN NOROXIA AND HYPOXIA IN PATIENTS WITH HISTORY OF HAPE AND AMS

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Cardiopulmonary responses to hypoxia at rest and during exercise were examined in patients with HAPE and patients with AMS compared to controls (C). Methods: 8 patients with history of HAPE, 6 patients with AMS and 8 controls performed incremental bicycle-exercise for 5 min at 25% V_Omax and 50% V_Omax at normoxia (N). At rest and in the 5th min of each work load the subjects rebreathed a gas mixture (0.4% C¹⁸O, 0.4% C₂H₂, 5% He, 5% SF₆, 5% CO₂, 35% O₂, rest N₂, volume 60% of IVC). Respired gas was analyzed by mass spectrometer. After a break the subjects inhaled a hypoxic gas mixture with 14% O₂ for 20 minutes. Under these hypoxic conditions (H) the test was repeated again. The dead space quotient (V_D/V_T) was calculated from the CO₂ endotriogram. Cardiac output (Qc) was calculated from C₂H₂ washout curves, pulmonary diffusion capacity (D_L) from the slope of log-transformed CO concentrations.

Results: Heart rate was significantly (p<0.05) higher during exercise in H. Qc was higher in hypoxia in C. At 25% V_Omax Qc was slightly lower in AMS and HAPE and higher at 50% V_Omax in hypoxia compared to normoxia. There was no significant difference of AaDO₂ during exercise between N and H in HAPE, AMS and C. Pulmonary diffusion capacity was significantly (p<0.05) lower in HAPE compared to AMS and C. At the same VE, V_D/V_T was not different in H and N in all three groups.

Conclusion: After 20 min exposure to FIO₂ of 0.14 and during hypoxic exercise, alterations of pulmonary diffusion, but no alterations in V_D/V_T could be observed in HAPE-susceptible subjects.

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HYPOXIA-INDUCED ACTIVATION OF ENDOTHELIAL CELLS

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In response to hypoxemia, endothelial cells may release factors playing a role in the vasoconstrictor tone and capillary permeability. These phenomena are observed in physiological and pathophysiological conditions at high altitude. Markers of endothelium activation were monitored in two studies. (1): in 10 healthy volunteers, plasma endothelin 1 (ET1) was determined at rest and during exercise (40 and 70% of max. aerobic power) at sea level (normoxia, N1) and after one week (Hypoxia, H1) and 3 weeks (H2) at an altitude of 6,542m (Mt Sajama, Bolivia). (2): ET1 and plasma concentrations of adhesion molecules ELAM-1 and ICAM-1 were measured at rest in 5 healthy men, at sea level (N2) and after 5 days (H3) at 4,350 m (Obs. Vallois, France).

	ET1	ELAM-1	ICAM-1
Study 1 (n=10)	pg/ml	ng/ml	ng/ml
Normoxia, N1	5.7±0.9	/	/
Hypoxia, H1	8.6±2.4***	/	/
Hypoxia, H2	7.7±1.4**	/	/
Study 2 (n=5)			
Normoxia, N2	3.9±1.3	26±13	215±45
Hypoxia, H3	7.1±1.4*	37±17*	239±21

H vs N : *, p<0.05, **, p<0.01, ***, p<0.001

ET1 increased in all altitude conditions. ELAM-1 but not ICAM-1 increased at 4,350m. ET1 was lower in H2 than in H1 (p<0.001), indicating an acclimatization effect. ET1 did not increase with exercise in normoxia or in hypoxia. In conclusion, altitude hypoxia (4,350 and 6,542m) activates the endothelial cells. Shear stress is not likely a predominant factor for this activation.

With the support of ARPE-SANDOZ partnership.

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NIFEDIPINE PREVENTS HYPOXIA-INDUCED DECREASE OF Na,K-ATPASE ACTIVITY IN ALVEOLAR TYPE II CELLS

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Alveolar hypoxia may induce acute lung injury with pulmonary edema. The cationic sodium transport by alveolar epithelium represents an important mechanism for airspace fluid clearance in acute lung injury. In the present study, we examined whether hypoxia affects Na,K-ATPase activity in alveolar epithelial cells (AEC). SV40 virus transformed rat type II AEC were cultured on collagen-coated plastic dishes and exposed to either hypoxic (5% O₂) (H) or normoxic (21% O₂) (N) for increasing times (up to 48 h) in the absence or presence of 10⁻⁵ M nifedipine. Na,K-ATPase activity was determined using ouabain-sensitive ⁸⁶rubidium influx (OsRb). Exposure to hypoxic atmosphere for at least 12 h induced a time-dependent decrease of OsRb (101 ± 9.7 in H vs 218 ± 14 nmol/10 min/mg prot in N after 24 h; p<0.001). Cells exposed to H for 24 h decreased intracellular ATP by 25% compared to N cells. However, equivalent ATP depletion in N cells did not affect OsRb. Incubation of N cells with supernatant of H cells resulted in a 45% decrease of OsRb within 1 h. Nifedipine prevented hypoxia-induced decrease of OsRb. These results indicate that: 1) hypoxia induces a time-dependent decrease of Na,K-ATPase activity in alveolar type II cells, 2) this effect is most likely due to the release of a soluble factor and 3) is prevented by nifedipine. Autocrine alteration of AEC Na,K-ATPase activity during hypoxia may reduce airspace fluid clearance and contribute to the formation and/or maintenance of alveolar edema.

ABSTRACTS

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ROLE OF G PROTEINS IN THE HYPOXIA-INDUCED DESENSITIZATION OF CARDIAC BETA-RECEPTORS

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Prolonged exposure to high-altitude hypoxia induces a decrease in maximal exercise heart rate and in chronotropic response to endogenous (exercise) and exogenous (isoproterenol) adrenergic activation (Richalet et al., J. Appl. Physiol. 67:523, 1989; Antezana et al., J. Appl. Physiol., 76:1055, 1994). Desensitization of B-receptors (B-AR) could be one of the mechanisms involved. A hypoxia-induced decrease in density of B-AR was found in human lymphocytes and in rat myocardium. Chronic hypoxia also induced, on the left ventricle of rats, a downregulation of adenosine receptors and an upregulation of muscarinic receptors (Kacimi et al., J. Appl. Physiol. 75:123, 1993). All these effects could be linked to a modification in the synthesis and/or activity of Gs and G12 proteins. We examined the effect on gene expression and level and function of G proteins in the heart of rats exposed for 30 days to hypobaric hypoxia (H, 380 mmHg), compared to normoxic rats (N). Myocardial Gs and G12 mRNAs were identified by Northern blot and quantified by slot blot hybridizations. In right ventricles (RV) which were hypertrophied by hypoxia-induced pulmonary hypertension, mRNA levels of G12 were increased by 40% ($p<0.05$) without changes in left ventricles (LV). No change was observed in Gs mRNA levels of both RV and LV. Immunoblotting analysis did not show any change in the amount of G12 in both LV and RV during HX. Functional activity of Gs, measured by reconstitution using cyc- cells deficient in Gs, was significantly decreased in HX LV (N: 2.33 ± 0.11 vs HX: 1.92 ± 0.70 pmol/ug/10min, $p<0.05$) and in HX RV (N: 2.05 ± 0.15 vs HX: 1.62 ± 0.08 pmol/ug/10min, $p<0.01$). In conclusion, gene expression and protein level and activity of Gs and G12 do not show parallel variations in HX. Desensitization in HX appears as related to a decreased functional activity of Gs in spite of a normal transcriptional activity. In HX RV, although mRNA level of G12 increased, protein level was unchanged.

With the support of ARPE-SANDOZ partnership.

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INHIBITION OF B-ADRENERGIC STIMULATION IN HIGH ALTITUDE GUINEA PIGS.

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To elucidate whether the downregulation phenomenon is also a genotypical answer to the evolution of animals in hypoxic environments, the heart rate (HR) response to isoproterenol (B agonist; 0.93 mg/Kg) and the density of B-AR (Bmax) and affinity constants (Kd) were evaluated in the guinea pig (*Cavia porcellus*) native to high altitude (HA; 4300 m; n=8) and in controls (SL; n=8). HA animals have a mean resting HR of 192 ± 71 bpm while sea-level guinea pigs have one of 243 ± 19.5 bpm ($p<0.05$). Isoproterenol increased the HR in both groups, but in the high-altitude guinea pigs an addition of 60% of the sea-level dose should be given to have only 56% of the sea-level cardiac response. HA guinea pigs also showed a decrease in Bmax ($p<0.05$) and in Kd ($p<0.01$) in the left ventricle (Bmax (fmol/mg prot): HA=55.2 \pm 18.0, SL=74.0 \pm 15.4); Kd (pM): HA=21.2 \pm 9.1, SL=39.1 \pm 13.7). Bmax and Kd in slightly hypertrophied right ventricle have not shown any change. Our findings indicate that HA guinea pigs are much less dependent on B-sympathetic stimulation, and support the notion that the downregulation phenomena is a mechanism of myocardial regulation preserved even in the guinea pig, an animal which has been exposed to high altitude since approximately 35 million years, well before the Andes have arisen.

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CARDIOPULMONARY KINETICS DURING RECOVERY AFTER REPEATED SUPRAMAXIMAL EXERCISES IN HYPOXIA.

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To evaluate the effect of hypoxia on the recovery after an anaerobic exercise, 6 sprint-trained (ST) and 6 endurance-trained athletes (ET) performed two 20-s bouts of supramaximal cycle exercise (W1 and W2; Wingate test, with a 5-min passive recovery period in between, in normoxia (N) and acute hypoxia (H, $\text{FiO}_2 = 0.115$)

	Mean (W.kg ⁻¹)	EPOC (ml.kg ⁻¹)
W1	W2	W1
N	10.73 \pm 0.63	9.88 \pm 0.78 [§]
H	10.55 \pm 0.93	9.81 \pm 0.89 [§]

	W1	W2	W1
N	11.10 \pm 0.70	11.10 \pm 0.68 [†]	66.7 \pm 6.2 [†]
H	10.96 \pm 0.91	10.90 \pm 0.59 [†]	54.6 \pm 4.1 ^{**} [†]

[§]p<0.05 W2 vs W1 ^{**}p<0.01 H vs N [†]p<0.05 ET vs ST

In ST and ET, hypoxia had no influence on mean power output (Pmean) delivered during the first and the second 20-s bouts. Furthermore, for both groups, hypoxia changed neither heart rate nor mean arterial pressure kinetics during recovery periods. In ET, but not in ST, each hypoxic exercise induced a transient drop ($p<0.05$) in arterial oxygen saturation at the onset of recovery periods. In ST and ET, hyperventilation reached higher values ($p<0.05$) after W2 than after W1. Hypoxia did not affect the recovery ventilation. The excess post-exercise oxygen consumption (EPOC) calculated over the first 5-min of recovery was of the same magnitude after W1 or W2, but EPOC decreased with hypoxia. These data suggest that, in highly trained athletes, acute hypoxia does not interfere with the restoration of anaerobic power, despite a reduced systemic oxygen consumption during recovery in hypoxia.

With the support of ARPE-Sandoz partnership.

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HIGH ALTITUDE HYPOXIA AND CALCIUM METABOLISM.

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The effects of altitude hypoxia on bone markers, serum and urinary parameters of calcium metabolism and the biological response to a 200U 1-34 PTH injection were studied in 10 men (age: 23-49) during a 5 day stay at 4,350 m. All subjects were unacclimatized (no stay over an altitude of 1000m for the 3 preceding months) and received 2.5 mg (100,000 IU) vitamin D 6 weeks before. The results were compared to those obtained at sea level (normoxia), in basal conditions (before PTH injection) the following values were obtained (mean \pm SD):

	normoxia	hypoxia	
Ca ⁺⁺ (mM)	1.21 \pm 0.04	1.20 \pm 0.04	NS
PO ₄ (mM)	1.04 \pm 0.10	1.06 \pm 0.18	NS
25(OH)vitaminD (ng/ml)	26.8 \pm 6.3	26.9 \pm 6.3	NS
calcitriol (pg/ml)	36.4 \pm 10.0	48.0 \pm 18.0	***
PTH (pg/ml)	26.4 \pm 6.7	23.1 \pm 6.5	NS
Osteocalcin (OC) (ng/ml)	22.5 \pm 5.1	16.2 \pm 6.3	***
uCa/uCreat	0.34 \pm 0.15	0.23 \pm 0.13	NS
uPO ₄ /uCreat	2.27 \pm 0.91	0.56 \pm 0.38	***
PO ₄ reabsorption rate (%)	84.0 \pm 13.0	97.4 \pm 2.1	***
u cyclic AMP (nmol/lrn)	5.9 \pm 4.0	3.6 \pm 1.2	NS
u cross-laps (mg/mg urecreat)	0.12 \pm 0.12	0.13 \pm 0.18	NS

NS : p>0.05; *** : p<0.001

The magnitude of the response to exogenous PTH was similar in normoxia and hypoxia for serum Ca⁺⁺, PO₄, calcitriol, uCa/urecreat and PO₄ reabsorption rate but significantly lower in hypoxia for cAMP ($p<0.05$). We conclude that, in these conditions of hypoxia, a relative resistance to PTH occurs, the origin of which deserves further studies. Increase in calcitriol is unexplained. At the bone level we observed an osteoblastic depletion (shown by a decrease in OC) uncoupled with osteoclastic resorption (no change in u cross-laps excretion).

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URINARY LEUKOTRIENE LEVELS ARE ELEVATED UPON EXPOSURE TO HIGH ALTITUDE AND CORRELATE WITH SYMPTOMS OF ACUTE MOUNTAIN SICKNESS.

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Circulating eicosanoids have been reported to increase upon rapid ascent to high altitude, suggesting a role in the etiology of Acute Mountain Sickness (AMS). We measured urinary leukotriene E_2 (ULTE $_2$) levels in eight healthy men at sea level (SL), and 36 hours after final ascent to 4300m (HA), following four days residence at 1830m. AMS symptoms were assessed using the Environmental Symptoms Questionnaire (ESQ), with indices derived from the ESQ weighted towards cerebral (AMS-C) and respiratory (AMS-R) symptoms. The mean SL ULTE $_2$ level (\pm SEM) was $67.9 (\pm 37.3)$ pg/mg creatinine (cr), and the mean HA level was $134.8 (\pm 19.4)$ pg/mg cr ($p<0.01$). HA ULTE $_2$ correlated with HA AMS-C (mean score 0.876, $r = 0.76$, $p<0.03$) and HA AMS-R (mean score 0.525, $r = 0.71$, $p < 0.05$). We conclude that ULTE $_2$ levels are elevated upon exposure to HA even after staging for four days at 1830m, and that these values correlated with AMS symptoms. This study supports the hypothesis that leukotrienes are involved in the pathophysiology of AMS.

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EFFECTS OF PREGNANCY ON VASCULAR ENDOTHELIAL SMOOTH MUSCLE ACTIVITY: LOW ALTITUDE STUDIES.

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Pronounced vasodilation of the maternal vasculature and attenuated pressor responsiveness to infused vasoconstrictors occurs during normal pregnancy, whereas, during preeclampsia many of these responses are reversed. Because hypobaric hypoxia is associated with an increased incidence of preeclampsia, we investigated the contributions of endothelial-dependent and -independent alterations in vaso-dilator and -constrictor responses in 10 normotensive, nulliparous women at wks 24 and 36 of pregnancy and 3 mo postpartum residing at 1600 m. These women will be compared with subjects residing at 3100 m. No vasoconstriction was observed in response to intra-arterial infusion of either phenylephrine (0.1-10.0 μ g/min) or angiotensin II (0.01-0.3 μ g/min) during pregnancy at dosages shown to produce vasoconstriction in nonpregnant women. There was a progressive rise in forearm vascular resistance during acetylcholine infusion (0.03-10.0 μ g/min/100 ml) from wk 24 to 36 of pregnancy. However, the % relaxation was not different (A baseline-max dose=90% at wk 24 vs 94% at 36). A similar decline in forearm vascular resistance during sodium nitroprusside infusion (0.3-0.6 μ g/min/100 ml) occurred but again, the % relaxation was the same. A serial increase in Von Willebrand factor antigen, a marker of endothelial cell function, occurred from wk 12 to 36 of pregnancy. These findings suggest that a progressive fall in forearm vascular resistance is accompanied by greater endothelial-dependent and -independent vasodilator activity. However, these vasodilatory changes are dissociated from a pregnancy-associated blunting of vascular smooth muscle vasoconstrictor responsiveness. Thus, both endothelial and vascular smooth muscle cell alterations may contribute to the normal vascular adjustments to pregnancy and possibly their alteration during hypobaric hypoxia associated preeclampsia.

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ACUTE MOUNTAIN SICKNESS AND VENTILATORY PATTERN AT REST AND MAXIMAL EXERCISE IN SUBJECTS INTERMITTENTLY EXPOSED TO HYPOBARIC HYPOXIA.

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Acute mountain sickness (AMS) is a transient and self-limiting syndrome that differs among individuals and deteriorates the work capacity at high altitude. Whether subjects with AMS have an impaired ventilatory response to hypoxia is controversial. We evaluated if AMS symptoms correlate with respiratory parameters at rest and during maximal exercise in humans exposed to hypobaric hypoxia. We studied 23 healthy males ($\bar{x} = 38.9$ years) exposed repeatedly to 4600 m altitude, with intervals of residence at sea level. During the first day at high altitude, heart rate (HR), O_2 arterial saturation (O₂sat) and ventilatory pattern at rest and maximal exercise were measured during treadmill exercise (Bruce protocol). The magnitude of AMS symptoms was quantified with the Lake Louise Consensus Questionnaire elaborated at International Hypoxia Symposium of 1991. Analysis between AMS score and ventilatory pattern were done by Pearson correlation coefficient. $P < 0.05$ was considered significant.

Results:

	mean \pm SD	r
O ₂ sat rest (t)	87.0 \pm 1.1	0.21
O ₂ sat max exerc. (t)	77.3 \pm 1.9	0.31
VE rest (l/min)	13.3 \pm 0.8	0.05
VE max (l/min)	124.6 \pm 6.4	0.24
V _e rest (l)	0.86 \pm 0.05	0.26
V _e max (l)	3.10 \pm 0.08	0.32
RR rest (x')	16.5 \pm 0.7	0.34
RR max (x')	40.8 \pm 2.1	0.35
V _{O2} maximo (l/min)	3.96 \pm 0.15	0.31 N.S.

We conclude that ventilatory performance at rest and maximal exercise in subjects exposed to hypobaric hypoxia didn't correlate with AMS symptoms (score average: 4.8 \pm 0.3). Supported by Sociedad Minera Collahuasi.

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THE INSPIRATORY MUSCLES FUNCTION IN SUBJECTS EXPOSED INTERMITTENTLY TO HYPOBARIC HYPOXIA.

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To assess the effect of intermittent exposure to hypobaric hypoxia on inspiratory muscle performance we studied 51 subjects (range 31 \pm 8.6 years) who worked in a gold mine in the north of Chile using a schedule of 8-12 days of work at 4600 m altitude followed by 4 days of rest at sea level. The acute mountain sickness symptoms were evaluated through a questionnaire elaborated in the International Hypoxia Symposium of Canada in 1991. Maximal inspiratory pressure (P_{Max}) was measured as an index of strength. Endurance was evaluated through the maximal inspiratory load (SIL) and the maximal inspiratory pressure (SIP) they could sustain for 2 minutes. Studies were performed at sea level (A), in the first two days of ascending (B) and in the 3 or 4 days of staying at 4600 m (C). Analysis were done by ANOVA for repeated measures and Newman Keuls student t test.

Results ($\bar{x} \pm SD$):

	A	B	C
P _{Max} (cmH2O)	139 \pm 18	124 \pm 15 (*)	130 \pm 18 (*)
SIL (g)	571 \pm 13	498 \pm 98 (*)	544 \pm 106 (*)
SIP (cmH2O)	107 \pm 21	95 \pm 16 (*)	103 \pm 18 (*)

* $p < 0.01$

Results showed significant differences in all of the indices in the three conditions. The lower values observed in B could be explained by a direct effect of hypoxia on strength and endurance and/or to the acute mountain sickness symptoms presented by the subjects in this condition (score average: 6.4 \pm 3.1). The lower values in condition C as compared to A, may be explained by the effect of hypoxia.

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ABSTRACTS

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ACUTE MOUNTAIN SICKNESS AND VENTILATORY FUNCTION IN SUBJECTS INTRIMENTTINELY EXPOSED TO HYPOBARIC HYPOXIA.

Saldías F, Beroizta T, Jalil J, Casanegra P, Lisboa C. Departments of Respiratory and Cardiovascular Diseases, Catholic University of Chile. Maroleta 345, Santiago.

To assess the magnitude of the symptoms of acute mountain sickness (AMS) and the ventilatory function in subjects intermittently exposed to hypobaric hypoxia, we studied 48 healthy men (range 32.6 ± 8.2 years) who worked in a gold mine at an altitude of 4,600 m, using a schedule of 8-12 days of work in the mine followed by 4 days of rest at sea level (Arica). Studies were performed in Arica (A), in the first two days of ascending (B) and after 3 or 4 days of staying at 4600 m (C), with a Collins Eagle II spirometer, following the American Thoracic Society recommendations. The AMS symptoms were evaluated by a questionnaire elaborated in the International Hypoxia Symposium of 1991. Analysis were done by ANOVA for repeated measures and Student *t* test for paired measures.

Results (X±SD):	A	B	A	C
FVC (l)	5.310.6	5.210.5 *	5.510.8	5.410.8
FEV1 (l)	4.310.5	4.410.6	4.410.7	4.510.7
PEF25-75 (l/s)	4.310.9	5.011.6 *	4.111.1	4.711.5 *
PEF (l/s)	9.411.5	11.411.9 *	9.811.3	11.512.3 *

* p<0.01

Small decrease in FVC occurred on arrival at altitude (B), probably secondary to an increase in pulmonary blood volume. The expiratory flows increase at 4600 m could be explained by the changes in the air density. The subjects reported mild to moderate AMS symptoms during the first 24 hours of ascending (score 6.4±1.1). There was no correlation between the magnitude of AMS and the changes in ventilatory function at altitude.

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ACCLIMATIZATION TO HIGH ALTITUDE : A BIOCHEMICAL EVALUATION

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Exposure to high altitude hypoxia causes a variety of biochemical and metabolic changes leading to tissue injury and imbalance in oxygen supply. Therefore, proper acclimatization to hypoxic stress is essential for the body to adapt to altered metabolic processes. A study on 68 subjects drawn from the Indian Army inducted to high altitude either by air (acute induction) or by road (gradual induction) was conducted to evaluate the acclimatization of the subjects on the basis of biochemical and metabolic changes. They were divided into two groups. Group A: air-inductees, air lifted to an altitude of 3,500m in 1h, and group B: road-inductees, transported to the same height in 4 days. Fasting blood was collected between 0600 and 0700h on arrival at sea level and then after arrival at an altitude of 3,500m on day 1, and 5 days later processed immediately for 2,3-DPG, cortisol, cholinesterase, glutamyl transferase, uric acid, cholesterol, HDL and LDL-cholesterol and triglycerides. Plasma cholesterol, LDL-cholesterol, triglycerides, 2,3-DPG, uric acid and cholinesterase levels were higher on day 1 in group B. In group A, these biochemical indices were higher till day 5 whereas in group B, these biochemical indices increased on day 1 only. The results demonstrated that the biochemical indices tend to reach towards sea level values from day 3 onwards in the case of road inductees. The biochemical and metabolic changes may be due to not only decreased oxygen consumption but also decreased metabolic demands.

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NEUROPATHOLOGIC FINDINGS IN MICE FOLLOWING EXPERIMENTAL NEONATAL HYPOXIC ENCEPHALOPATHY

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As we reported before, 1-day-old mice, Jcl:ICR strain, showed intracranial hemorrhage when they were subjected to hypoxia, 5% oxygen and 95% nitrogen mixture, for 8 hours, and survived up to adulthood. To investigate the evolution of the hypoxic brain injuries in the neonatal period, we examined the mouse brains periodically from the time of hemorrhage up to the 15th day. Symmetrical hemorrhage occurred suddenly in the bilateral parietal cortex between 1.5 and 4 hours after hypoxic exposure. Cysts were observed in the periventricular region, temporal cortex, external capsule and amygdaloid nucleus 1 to 2 days after hemorrhage, but were not found in the parietal cortex where hemorrhage occurred. The cysts in the periventricular region enlarged gradually up to the 3rd day, and then reduced in size. By the 7th day they had completely disappeared. The cysts in the temporal cortex and amygdaloid nucleus however, persisted even on the 15th day. These brain injuries following hypoxia correspond to the parasagittal cerebral injury, periventricular leukomalacia, porencephaly and multicystic encephalomalacia of human neonatal hypoxic ischemic encephalopathy. This study suggests that histopathological differences are important factors in the formation of these brain injuries following hypoxia.

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CARDIAC PARASYMPATHETIC CONTROL IN TIBETAN NEWCOMERS TO ALTITUDE (5050 M)

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The purpose of this study was to determine whether enhanced parasympathetic action contributes to the reduction in maximal heart rate (HR_{max}) in chronic hypoxia, in first-time, Tibetan sojourners to altitude. Studies were performed at low altitude (1300 m, LA) and after 1 month acclimatization to high altitude (5050 m, HA) in 8 Tibetan men (18-25 yr). The increase in HR_{rest} with atropine (1.8 mg i.v.) was greater (p<0.01) at LA than HA (LA: 65±7 (mean ± SD) to 102±9 b·min⁻¹, vs. 89±9 to 117±10 b·min⁻¹). At HA, HR_{rest} was reduced compared to LA (173±7 vs. 188±10 b·min⁻¹, p<0.001) but was not altered significantly by atropine (176±7 b·min⁻¹), whereas at LA it decreased slightly to 182±10 b·min⁻¹ (p<0.05). The atropine-induced attenuation of the slope of the HR vs. work rate relationship (p<0.001) was similar between LA (33%) and HA (34%), even though the slope was reduced at HA compared to LA (p<0.001). These findings suggest that enhanced cardiac vagal control does not contribute to the lowered hypoxia-induced HR_{max} in Tibetan newcomers to altitude (5050 m).

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BODY FLUID SHIFTS DURING ACUTE HYPOBARIC HYPOXIA
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Acute hypobaric hypoxia (AHH) leads to hemococentration and a decrease in plasma volume (PV). In order to investigate the role of fluid shifts from peripheral to central compartments during AHH the changes in lower limb (LV) and forearm volumes (FV) were plethysmographically measured in 10 healthy volunteers exposed to graded AHH in a hypobaric chamber. Total altitude exposure period of the stepwise ascent and descent (450m-2500m-3500m-4500m-2500m-450m) lasted 2hrs. During ascent a decrease both in LV and FV of -0.52 ± 0.39 ml/100ml (mean \pm SD) and -0.65 ± 0.32 ml/100ml respectively was found. However, during descent LV shows a further small, but not significant decrease (-0.02 ± 0.11 ml/100ml), whereas FV tends to increase slightly ($+0.03 \pm 0.13$ ml/100ml). During the whole AHH exposure PV decreased ($-4.66 \pm 2.95\%$) and urine volume increased (0.84 ± 0.41 to 3.29 ± 1.43 ml/min). We conclude that the decrease in LV and FV under AHH reflects a blood volume shift from peripheral to central compartments and that the decrease in PV might be due to the elevated diuresis and not to an enhanced filtration of intravascular fluid into tissue.

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EFFECT OF β -BLOCKADE ON CARDIAC VOLUMES IN
HEALTHY MEN AT HIGH ALTITUDE

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The purpose of this investigation was to determine the effect of β -adrenergic blockade on left ventricular volumes and function during exposure to 4300 m (USARIEM Pikes Peak, CO). Eleven male sea level residents were divided into control and drug groups and given either placebo (CONT) or propranolol (DRUG) 240 mg/day, respectively. β -blockade was confirmed by a blunted chronotropic response to isoproterenol. Echocardiography was performed on supine resting subjects at sea level (SL), and after 1 (HA 1) and 21 (HA 21) days at altitude. End-diastolic (EDV) and end-systolic (ESV) volumes were calculated from single-plane area length measurements in the apical 4 chamber view. Ejection fraction (LVEF) was equal to EDV - ESV/EDV. Results were mean \pm SEM. (* vs SL, p < 0.05, ANOVA).

CONT DRUG

	SL	HA 1	HA 21	SL	HA 1	HA 21
HR (bpm)	73 \pm 5	101 \pm 7*	86 \pm 6	55 \pm 3	75 \pm 4*	68 \pm 5*
EDV (ml)	90 \pm 6	95 \pm 5	75 \pm 7	100 \pm 5	97 \pm 5	79 \pm 7*
ESV (ml)	31 \pm 4	28 \pm 3	30 \pm 5	27 \pm 2	25 \pm 2	26 \pm 2
SV (ml)	60 \pm 3	67 \pm 5	45 \pm 5*	73 \pm 3	71 \pm 3	53 \pm 5*
LVEF (%)	67 \pm 3	72 \pm 1	60 \pm 4	73 \pm 1	74 \pm 2	67 \pm 2*

The change (Δ) in resting HR at 4300 m compared to SL was independent of β -blockade. Stroke volume decreased over time at 4300 m, independent of β -blockade, primarily related to decreases in EDV. Chronic hypoxia had minimal effect on resting LVEF, with no additional influence from β -blockade. In summary, the resting hemodynamic adaptations observed during a 21 day sojourn at 4300 m were unaffected by β -blockade. Supported by US Army and NIH HL-14985

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EFFECT OF HIGH ALTITUDE ON CIRCULATING
LEVELS OF THE CYTOKINE TUMOR NECROSIS
FACTOR- α IN HAPE-SUSCEPTIBLE SUBJECTS

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The purpose of this investigation was to determine whether subjects susceptible to high altitude pulmonary edema (HAPE), a type of permeability lung edema, had increased levels of tumor necrosis factor (TNF) during exposure to 4400 m. Eight male subjects, aged 20-37 years, 4 with HAPE-S and 4 without (CONT) a history of HAPE, were decompressed in a hypobaric chamber for up to 8 hrs. AMS symptom questionnaires (ESQ), echocardiograms, and blood samples were obtained at 1500 m and repeated hourly at 4400 m. Mean pulmonary artery pressure (MPAP) was calculated from the ratio of acceleration time (ACT) to ejection time (RVEI), $MPAP = (0.55 \cdot ACT/RVEI)/0.0055$, using the Doppler flow signal from the right ventricular outflow tract. TNF measurements using a commercially available ELISA (Endogen, Boston, MA) were performed on plasma treated with aprotinin (normal value < 100 pg/ml).

	ESQ	MPAP (mmHg)	TNF (pg/ml)
4 hr	pre	4 hr	pre
CONT	0.3 \pm 0.1	16 \pm 1	37 \pm 2*
HAPE-S	1.0 \pm 0.4	21 \pm 3	47 \pm 6†

* vs pre, † vs CONT, p < 0.05. Two of the 4 HAPE-S subjects met criteria for the diagnosis of HAPE at 4 hours, prompting their removal from the chamber. In summary, the HAPE-susceptible subjects developed greater symptoms of AMS and more severe pulmonary hypertension, confirming their increased susceptibility to altitude sickness, but did not have elevated levels of the cytokine TNF.

Research supported by NIH Grant HL-14895

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INTERMITTENT HYPOXIC TRAINING AND THE INDIVIDUAL
RESPONSE OF RESPIRATION AND FREE RADICAL PROCESSES

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Recent investigations on human twins has shown that the ventilatory response to hypoxic stimulus (HVR) is a rigid genetically determined physiological characteristic, reflecting an individual's overall nonspecific reactivity. We have examined the relationships between HVR and its influence on free radical processes and some indices of antibacterial defense system during adaptation to periods of intermittent hypoxia. All 31 men resided at sea level, ranged from 20 to 48 years old, and were exposed to normobaric isocapnic progressive hypoxia (individualized rebreathing technique starting from room air up to individual tolerable levels: 50 to 35 mmHg range) during 15 days of three daily 5-7 min. sessions with 15 min breaks. This hypoxic training (HT) caused a group increase in: (1) HVR slope of 42 ($\pm 2.9\%$), (2) tolerance to extreme hypoxia of 18 ($\pm 1.4\%$) and (3) forced expired volume of 21 ($\pm 1.7\%$) in all subjects. After HT there was a decrease in both spontaneous and initiated chemiluminescence (ChL) by 24 ($\pm 2.2\%$) and 26 ($\pm 2.8\%$) respectively accompanied by a decrease in malon dialdehyde (MDA) concentration of 21 ($\pm 3.9\%$). The change in (Δ) parameters of ChL, MDA, and leucocyte enzyme activity (LEA) were to be closely related to HVR. The higher the pre-HT HVR value, the greater the Δ the experimental variables of ChL, MDA and LEA. The 5 men with lowest pre-HT HVR values actually had a slight increase in ChL and MDA values post-HT. As a group the men demonstrated greater activation of NADPH-oxidase (NPO) and myeloperoxidase (MPO) activities of neutrophils. Correlation coefficients between initial HVR and Δ other parameters were: Δ ChL, $r=0.68$; for Δ MDA, $r=0.54$; for Δ NPO, $r=0.76$; for Δ MPO, $r=0.64$. Thus, we suggest that intermittent hypoxic training has a noticeable positive effect upon respiration and free radical processes which varied in degree with each individual but was uniformly beneficial in direction.

ABSTRACTS

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HYPOTHETIC ROLES OF OSMOTIC PRESSURE AND ANGIOGENESIS IN HIGH ALTITUDE CEREBRAL EDEMA

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High altitude cerebral edema (HACE) has been attributed to ion pump failure from ATP depletion (Houston) or high cerebral blood flow and/or high intracranial pressure (Sutton, Lassen, Krasney). However, ATP remains normal even after hypoxically-induced neuronal silence, and CO_2 hyperemia does not cause cerebral edema. Two new hypotheses are: 1) Severe hypoxic substrate-induced osmotic cell swelling: ADP rise (Pasteur eff.) stimulates glycolysis, normalizing V_O_2 at lower a_a , P_O_2 by increasing all osmotically active acid anion substrates. Cellular and mitochondrial osmotic pressure may rise 30 mOsm potentially increasing cell volume 10%. 2) Angiogenesis: Tissue hypoxia (Knighton, *Surgery* 90:262, 1981) and lactate ion (Jensen, Hunt, *Lab Invest* 54:574, 1986) are the key initiating factors which activate macrophages to up-regulate VEGF (vascular endothelial growth factor) and other enzymes (tFN α , IL-2 and 8, TAF, bFGF, GM-CSF, IGF-I, PDGF). These first degrade the extracellular matrix, permitting leakage of plasma and blood through capillaries, to promote endothelial cellular budding and tube formation. This may underlie the petechial hemorrhages seen in retinal nerve cell layers in mountaineers. Similar pathology may occur throughout the brain. Plasma and blood leakage activate coagulation which may account for venous thromboses seen at autopsy. Dexamethasone is the most effective drug shown to prevent and treat HACE. Its effect is limited to reduction of cerebral edema signs and symptoms. It is also the best known blocker of angiogenesis *in vivo* and *in vitro*. Planned experiments will test hypoxic rabbit CSF for VEGF *et al* with and without dexamethasone pretreatment.

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ABSENCE OF LUNG IMPEDANCE RESPONSE TO UNILATERAL HYPOXIC VENTILATION IN INTACT DOGS

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The effects of hypoxia on lung and airway mechanics remains controversial. We examined the effect of alveolar hypoxia (without systemic hypoxemia) in three intact, anesthetized mongrel dogs by measuring individual lung respiratory system impedance from 0.2-2 Hz while independently ventilating the left (L) and right (R) lungs. Lung isolation was obtained with a Kottmeier double lumen endobronchial tube modified for distal pressure measurement at the bronchial opening. Individual lung input impedance (Z) was measured during sinusoidal forcing with 45 ml volume at frequencies (f) of 0.2, 0.5, 0.8, 1.4 and 2.2 Hz during control (90% O_2 to both lungs) and hypoxic (O_2 8% left, 90% right) ventilation. Tidal volume was adjusted to keep end tidal PCO_2 constant in both lungs. Systemic arterial $\text{PO}_2 / \text{PCO}_2$ (mean \pm s.e.) was $325\pm24 / 27\pm5$ mmHg during control and $158\pm38 / 31\pm1$ mmHg during hypoxic ventilation. There were no significant changes in L or R impedance magnitude $|Z|$, the real or imaginary components of Z , or the L/R ratio of $|Z|$ at any f after 10 minutes of hypoxic ventilation of the L lung. Administration of 0.2 mg/kg of atropine i.v. slightly decreased the control impedances but did not alter the lack of response to hypoxia. We conclude that in intact dogs unilateral hypoxic ventilation without systemic hypoxemia does not alter lung mechanical properties.

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EFFECT OF CHRONIC EXPOSURE TO HIGH ALTITUDE HYPOXIA ON FEEDING BEHAVIOUR AND HEDONIC MATRIX.

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The armed forces personnel have to operate in different stressful environments like high altitude (HA) and they should be in a good mental and physical state in order to carry out specific tasks assigned to them. The commonest complaint of the soldiers posted at high altitude are hypophagia and anorexia. Hypophagia may be leading to the changes in taste sensitivity, since the taste system does more than identify chemicals in oral cavity. The present series of experiments were designed to investigate the effect of chronic HA hypoxia on feeding behaviour and gustatory responses. Albino male rats were exposed to a simulated HA equivalent to 7620 m continuously for 18 days in a decompression chamber. The rats were brought to sea level each day for 2-3 hours for recouping their 24-h food & water intake, body weight, and for carrying out one bottle test for recording their gustatory responses. Blood samples were drawn once a week from the retro-orbital venous plexus for blood sugar analysis. All the parameters were recorded before, during and after exposure to HA. The results showed a decrease in 24-h food and water intake and body weight during HA exposure. The blood sugar reflected a state of mild hypoglycemia. Single choice taste solution test showed an increased preference for sweet solution (glucose 13%, saccharine 0.2%) over citric acid, sodium chloride (0.9%) and quinine sulphate (0.001%) during exposure to simulation HA. Glucose intake showed a marked significant increase from 4th day onwards during HA exposure period. Saccharine intake was significantly higher on 5th days onward as compared to the corresponding pre-exposure phase. The details of the results will be discussed. However the actual intake of glucose was more (five times its pre-exposure) than saccharine during exposure time. Sodium chloride, citric acid and quinine sulphate did show an increase during the exposure period as compared to pre-exposure period but this increase was not found to be significant statistically. Preference for sweet solution over other taste modalities indicate that the sensory signals are important cues in the control of food intake under nutritional stress like hypophagia caused by HA exposure.

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CALCIUM AND POTASSIUM CHANNEL REGULATION AND RELATED CHANGES IN VASCULAR SMOOTH MUSCLE CONTRACTILITY AT HYPOXIA

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The present study was designed to examine the effect of hypoxia on Ca^{2+} and K^+ permeability in plasma membrane and related contractile responses of rat portal vein. It was shown that gradual decrease in bath P_O_2 below 14.3 - 13 kPa resulted in the inhibition of spontaneous myogenic activity and relaxation of vascular smooth muscle (VSM). The similar effects were produced in VSM by a Ca^{2+} -free solution and Ca^{2+} channel blockers. ^{45}Ca uptake was decreased at hypoxia. Hypoxia led to decrease of peak amplitude of Ca^{2+} current, increased the transient and oscillatory component of Ca^{2+} -activated K^+ current and was able to increase the amplitude and frequency of spontaneous transient outward potassium currents. Intracellular "injection" of phosphocreatine, but not adenosine triphosphate, to VSM cell myoplasm using liposomes prevented the inhibitor effect of hypoxia on VSM contractility. Thus, hypoxia may affect Ca^{2+} permeability and hence the VSM contractility for at least two mechanisms: i) by the decrease of a number of open Ca^{2+} channels as a result of the damage in their phosphorylation; ii) by Ca^{2+} channel inactivation resultant from a rise in intracellular Ca^{2+} which could be induced by a failure of active Ca^{2+} outward transport or/and intracellular Ca^{2+} sequestration.

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BRAIN GLUCOSE METABOLISM IN QUECHUAS AND SHERPAS

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The brain of hypoxia tolerant vertebrates is known to survive extreme limitations of oxygen, in part because of very low rates of energy production and utilization. To assess if similar adaptations may be involved in humans during hypoxic adaptation over generational time, volunteer Quechuas and Sherpas, indigenous to the Andes and to the Himalayas between about 3300 and 4900 m altitude, served as subjects in positron emission tomographic (PET) measurements of brain regional glucose metabolic rates. Two metabolic states were analyzed: (a) the presumed normal state (high altitude adapted) monitored as soon as possible after leaving the Andes and (b) the deacclimated state monitored after three weeks at low altitudes. Proton nuclear magnetic resonance spectroscopy studies of the Quechua brain found normal spectra, with no indication of any unusual lactate accumulation; in contrast, in hypoxia tolerant species, a relatively large fraction of the glucose taken up by the brain is released as lactate. PET measurements of [¹⁸F]-2-deoxy-2-fluoro-D-glucose (FDG) uptake rates, quantified in 26 regions of the brain, indicated systematically lower region-by-region glucose metabolic rates in Quechuas than in lowlanders. The metabolic reductions were least pronounced in primitive brain structures (eg cerebellum) and most pronounced in regions classically associated with higher cortical functions (eg frontal cortex). Fully analogous studies of sherpas showed no systematic differences from lowlanders and no deacclimation effects. At this point it is unclear if the differences between the two altitude groups are due to different degrees or to different strategies of hypoxia adaptation. Supported by NSERC (Canada) and NSF (USA).

PHOSPHATIDYLCHOLINE LIPOSOMES POSSESS THE ABILITY TO SUPPORT VASCULAR SMOOTH MUSCLE CONTRACTILE FORCE UNDER HYPOXIA.

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The study was undertaken to determine the effects of phosphatidylcholine (PC) liposomes on contractility of the isolated segments of the rat portal vein under hypoxia. It was shown that PC liposomes when added to the bath solution in concentration 10-100 mcg/ml effectively prevented the inhibition of VSM myogenic activity under hypoxia (pO_2 - 3.9 kPa) and restored the decreased amplitude of phasic contractions of VSM being added to hypoxic solution. They are also reduced the degree of hypoxic relaxation of VSM preactivated with K^+ -rich solution and promoted an increase in the level of tonic tension developed by K^+ -activated VSM in hypoxic solution. In conclusion, antihypoxic effect of PC liposomes was suggested to be due to not only the modification of the phospholipid environment of calcium channels and related increase in calcium permeability of VSM plasma membrane but also may be determined by the defence of calcium channels against lipid peroxidation products action. It is probably that fatty acid from liposomes can be used for ATP production in VSM during hypoxia.

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VENTILATORY RESPONSE TO HYPOXIA DURING REST AND EXERCISE BEFORE AND AFTER A HIMALAYA EXPEDITION

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Ventilatory response to transient hyperoxia during hypoxia acc. to Dejours's technique was examined before and after an expedition (56 days, 30 days at 4900 m or higher).

Methods: 10 mountaineers were exposed to simulated hypoxia ($F_{iO_2} = 0.12$, 86 mmHg) before (I) and 3 days (n=4) and 8 days (n=6) after return from basecamp (II). After 25 min acclimatization, bicycle ergometer exercise was performed with increments of 50 W and 5 min. Respirated gases and ventilation (V_E) were analysed breath-by-breath by mass-spectrometer (AMS2000), pO_2 and SO_2 were measured in capillary blood. Ventilatory response was tested by administration of 2 breaths of 100% oxygen in rest and in the 4th min of a work load, the switching between F_{iO_2} 's of 0.12, 1.0 and 0.12 was imperceptible by the subjects. The concomitant decrease in V_E was calculated (ΔV_E).

Results: ΔV_E in rest ($F_{iO_2}=0.12$) was 42.2 (I) and 41.5 % (II) of V_E (n.s.), ΔV_E during exercise was approx. 32 (I) and 38 % (II) (n.s.). However, ΔV_E increased with decreasing SO_2 during exercise. $\Delta V_E/SO_2$ during exercise was higher after the expedition ($F=5.8$, $p<0.019$). Therefore, the ventilatory sensitivity ($\Delta V_E/(100-SO_2)$) at 150 W increased after the expedition from 0.87 ± 0.38 to $1.91 \pm 0.51 \text{ l min}^{-1} SO_2^{-1}$ ($F=2.45$, $p<0.01$).

Conclusion: After 3 and 6 days return from 30 days of high altitude exposure, increased ventilation sensitivity to oxygen was observed during exercise but not during resting conditions.

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PHOSPHATIDYLCHOLINE LIPOSOMES POSSESS THE ABILITY TO SUPPORT VASCULAR SMOOTH MUSCLE CONTRACTILE FORCE UNDER HYPOXIA.

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The study was undertaken to determine the effects of phosphatidylcholine (PC) liposomes on contractility of the isolated segments of the rat portal vein under hypoxia. It was shown that PC liposomes when added to the bath solution in concentration 10-100 mcg/ml effectively prevented the inhibition of VSM myogenic activity under hypoxia (pO_2 - 3.9 kPa) and restored the decreased amplitude of phasic contractions of VSM being added to hypoxic solution. They are also reduced the degree of hypoxic relaxation of VSM preactivated with K^+ -rich solution and promoted an increase in the level of tonic tension developed by K^+ -activated VSM in hypoxic solution. In conclusion, antihypoxic effect of PC liposomes was suggested to be due to not only the modification of the phospholipid environment of calcium channels and related increase in calcium permeability of VSM plasma membrane but also may be determined by the defence of calcium channels against lipid peroxidation products action. It is probably that fatty acid from liposomes can be used for ATP production in VSM during hypoxia.

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EFFECT OF INCREASED DIETARY CARBOHYDRATE ON SYMPTOMS OF ACUTE MOUNTAIN SICKNESS AND CIRCULATING CYTOKINES

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We investigated whether a high carbohydrate (CHO) diet reduces symptoms of acute mountain sickness (AMS); and whether circulating cytokines rise with hypoxia and correlate with AMS severity. Nineteen men and women ate isocaloric diets either high in CHO (68%) or of normal CHO content (45%) for four days. On the last day of each diet they were exposed eight hours of 10% normobaric oxygen. Each subject completed the Lake Louise Consensus Questionnaire (LLCQ) at the beginning and end of each hypoxic exposure, at which times plasma samples were assayed for the following cytokines: IL-1 β , IL-6, IL-8 and TNF- α . The basal respiratory quotient (mean \pm SD) rose with the high CHO diet (0.94 ± 0.04 vs 0.87 ± 0.03). AMS severity did not differ between the high and normal CHO diets (LLCQ scores: high CHO = 10.1 ± 3.8 vs. normal CHO = 10.3 ± 4.1). The measured cytokines did not change significantly during hypoxia, nor did they correlate with AMS severity. We conclude that a four day high carbohydrate diet does not reduce symptoms of AMS, and plasma IL-1 β , IL-6, IL-8 and TNF- α concentrations do not rise in AMS and thus are likely not mediators of this syndrome.

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SOME MECHANISMS OF INTERVAL HYPOXIC TRAINING EFFECTS IN CLINICAL MEDICINE

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Interval hypoxic training (IHT) can positively change the course of some diseases: chronic obstructive pulmonary diseases, primary hypercholesterolemia, stable angina pectoris, rheumatoid arthritis ,etc. On the apparently healthy volunteers (men, 22 years mean age) and in experiments (mail Vistar rats) some effects of IHT(10% 02 -60 min daily-20 days) were studied, using biochemical, physiological and morphological parameters. The significant increase of antioxidant enzymes activity was noted in rat: superoxide dismutase activity increased by 30% in blood and by 25% in brain. The cross sectional capillary area enlargement in the myocardium and in the brain cortex and capillary net volume in the myocardium were observed. After ligation of coronary arteries in rats, adapted to hypoxia, we discovered 60% decrease rate of ischemic and reperfusion arrhythmias , compared to control. During the hypoxic mixture inhalation (volunteers ,SaO₂ -76-80 %) the individual biochemical parameters changes were showed (in average the thyroxine blood level decreased by 30 %, ethanol level by 50 % and lactate by 20% , in the time of urinary acid and pyruvate levels increased by 18 % and 36 %). Thus, the short hypoxic periods prove the biochemical, physiological and morphological changes which may be the basic for IHT effects,

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THE IMPORTANCE OF PHOSPHOCREATINE DURING MAXIMAL INTERMITTENT CYCLING

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This study examined the importance of phosphocreatine (PCr) degradation in maintaining power output during 3 bouts of intermittent cycling. 7 healthy males completed 3 bouts of maximal isokinetic cycling (30 s, 100 rpm) with 4 min of rest between bouts. Total work production was calculated for each leg in 3 s intervals. Following bout 2, blood flow to one leg was occluded by cuffing the thigh (>250mmHg) during the rest period to prevent PCr resynthesis. Muscle biopsies were obtained from the vastus lateralis just prior to bout 3 from the cuffed (CUFF) and uncuffed (CONT) legs. The cuff was then released, bout 3 was completed and biopsies were immediately sampled from both legs. The total work produced by each leg was similar during bouts 1 (9.3, 9.6 kJ) and 2 (8.1, 8.3 kJ). PCr resynthesis was prevented by cuffing as PCr prior to bout 3 was 20.7±7.7 and 63.0±3.0 mmol/kg dry muscle in CUFF and CONT. Cuffing significantly reduced the recovery of muscle lactate (CUFF, 132.1±6.7 vs. CONT, 103.1±5.5 mmol/kg), H⁺ (287±26 vs. 217±15 nM), acetyl-CoA, ADP, and AMP but had no effect on ATP, glucose 6-phosphate, glycerol 3-phosphate and pyruvate. PCr degradation during bout 3 was 3.1 and 47.5 mmol/kg in CUFF and CONT. Changes in all other metabolites during bout 3 were not different between legs. Total work in bout 3 was reduced by 15% in CUFF (5.8 kJ) vs. CONT (6.8 kJ). The work produced in the 3 s time periods over the first 18 s was significantly reduced in the CUFF leg, while no differences existed in the last 12 s. The results suggest that PCr continues to be an important source of ATP resynthesis during repeated bouts of maximal cycling, as it provided ~15% of the total ATP required in bout 3. The majority of this ATP was provided during the initial 15-18 s of cycling. However, it is possible that the cuffing procedure increased blood flow and oxidative metabolism, leading to an underestimation of the importance of PCr.

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INHALED NITRIC OXIDE FOR HIGH-ALTITUDE PULMONARY EDEMA

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Pulmonary hypertension is a hallmark of high-altitude pulmonary edema (HAPE), and may contribute to its pathogenesis. We studied effects of inhaled nitric oxide (NO) on pulmonary artery pressure and arterial oxygenation in eight HAPE-prone and six HAPE-resistant mountaineers both in a high altitude laboratory (4559 m), and during hypoxic breathing at low altitude. At high altitude, the severity of pulmonary hypertension was directly related to oxygen saturation ($r=0.73$, $p=0.003$), and HAPE-prone subjects were markedly more hypoxicemic and had more pronounced hypoxic pulmonary vasoconstriction than controls. In HAPE-prone subjects, NO (40 ppm) evoked a 3.7-fold larger decrease in pulmonary arterial pressure than in control subjects (29±4 vs 8±4 mmHg, $p<0.01$), and improved arterial oxygenation (even though adding NO to the inspired gas led to an additional decrease in $\text{F}1\text{O}_2$). There was a direct linear relationship between the degree of pulmonary vasoconstriction and decreases in pulmonary pressure evoked by NO-inhalation ($r=0.89$, $p<0.0001$); and between the severity of hypoxia and changes in arterial oxygenation evoked by adding NO to the inhaled air ($r=0.79$, $p=0.001$). In contrast, in HAPE-resistant subjects at high altitude, and in both groups during acute hypoxic exposure at low altitude, NO-inhalation while also lowering pulmonary pressure, impaired arterial oxygenation. These findings suggest that during HAPE, but not during hypoxic pulmonary vasoconstriction alone, NO exerts beneficial effects on arterial oxygenation that may be related to its pressure lowering action.

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INTRACRANIAL PRESSURE, HIGH ALTITUDE AND ACUTE MOUNTAIN SICKNESS

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This study was to determine the role of raised intracranial pressure (ICP) in the pathogenesis of mild to moderate acute mountain sickness (AMS). Measurements of changes in ICP were made on 24 healthy subjects (22m:2f, aged 22-65 years) during the development of AMS on rapid ascent from 1348 to 5200m in the Himalaya. Changes in ICP were assessed by tympanic membrane displacement, measured at rest in lying and sitting positions; blood gases were measured in arterialised capillary blood and assessment of AMS by medical interview and questionnaire. No difference was found in the change in mean tympanic membrane displacement on ascending to 4120m or 5220m in subjects with or without AMS. Acute hypoxia at 3440m, without symptoms of AMS however was correlated with a rise in (ICP) equivalent to a mean rise of 93.1mm H₂O in ICP ($p<0.02$). Raised ICP, though temporarily associated with acute hypoxia, is not a feature of the early stages of AMS. The mechanism of high altitude cerebral oedema may not be caused by changes in intracranial pressure but due to other mechanisms.

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REFINEMENTS OF LAKE LOUISE SELF-REPORTING AMS QUESTIONNAIRE

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The Lake Louise (LL) and B.M.R.E.S. questionnaires were completed twice daily by 24 subjects, during a trek to 5200m over 7 days. The five LL questions scored 0-3 were compared with similar questions scored 0-4 in the more detailed BMRES questionnaire. There was close agreement on headache (LL vs BMRES headache at rest $r=0.94$) and GI symptoms (LL vs BMRES nausea and/or vomiting $r=0.86$) but less with GI symptoms and anorexia ($r=0.72$). LL fatigue/weakness and separate BMRES fatigue ($r=0.87$) and BMRES weakness ($r=0.88$) agreed but fatigue was often noted to be due to factors other than AMS. Difficulty in sleeping correlated in the two questionnaires ($r=0.89$) and the more precise LL wording was easier to complete. LL Dizzy/lightheadedness was used by 12 subjects and only on 1 or 2 occasions with mild symptoms. This question was not in the BMRES questionnaire. The LL 0-3 scoring system was satisfactory. It is suggested that the LL questionnaire is modified: a) GI symptoms are altered to 1. loss of appetite; 2. mild/moderate nausea; 3. severe nausea and/or vomiting. b) fatigue and/or weakness is altered by limiting the question to weakness. c) dizzy/lightheaded is omitted and another CNS symptom substituted.

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EFFECTS OF COMPOUND OF RHODIOLA CRENULATA ON EXERCISE PERFORMANCE DURING HYPOXIA

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The effects of compound of rhodiola crenulata(CRC) containing rhodiola crenulata and several kinds of other Chinese traditional and herbal drugs on exercise performance were investigated during hypoxia. On the 4th day of receiving CRC(drug group) or orally water(control group), 268 mice exposed to a simulated altitude of 10,000m and 20 rats exposed to a simulated altitude of 6000m were forced to swim in swimming pools. The survival time of mice was 73.2 ± 3.8 min in drug group and 60.5 ± 4.0 min in control group ($P<0.05$). The blood lactate in rats was 4.6 ± 1.8 mmol/L in drug group and 21.1 ± 1.8 mmol/L in control group ($P<0.05$). On the 10th day of receiving CRC orally or placebo, 10 sea-level healthy men performed an incremental exercise on cycle ergometer using cross-over design until exhaustion in hypobaric chamber at a simulated altitude of 4500m. FWC170 and anaerobic threshold(AT-determined by gas exchanges) in the subjects taking CRC were 11.6% and 17.0% higher than those in the subjects taking placebo($P<0.01$), respectively. These results indicated that CRC might play an important role in improving exercise performance during hypoxia.

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EFFECTS OF ACUTE HYPOXIA ON OXYGEN TRANSPORT IN TIBETAN AFTER LIVING AT SEA LEVEL FOR 4 YEARS

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To understand more physiological basic of the adaptational mechanism in Tibetan to hypoxia, the present study tries to investigate change in oxygen transport of young Tibetan men after a long period living at sea level. They had stayed in Shanghai for 4 years and did not go back to Tibet during this period. Other group of young Han men who were born in Shanghai were control group as Tibetan. Their arterial blood gas, arterio-venous difference in oxygen tension, (Radiometer ABL-3, Copenhagen) oxygen dissociation curve (Hemoxy-Analyzes TC5, U.S.A.) were observed at sea level and after 2h at 3700m (Hypobaric hypoxia). It was found at 3700m that Pao_2 and Sao_2 in Tibetan were more high than Han. Their value were respectively 7.2 ± 0.2 kpa, 5.5 ± 0.2 kpa ($p < 0.05$) and 87.9 ± 3.3 , 78.2 ± 1.6 % ($p < 0.05$). Arterio-venous difference in oxygen tension in Tibetan was longest than Han (Tibetan:4.0 kpa; Han:2.6kpa $p < 0.01$). $P50$ change in Tibetan and Han was 3.40kpa and 3.37kpa at sea level. At 3700m, $P50$ was 3.52kpa and 3.51kpa respectively. Hb concentration of Tibetan after a long period living at sea level were lower than those of Han (15.5 ± 1.6 g% and 16.3 ± 1.3 g%). Effects of acute hypoxia on their Hb were no change (15.1 ± 1.6 and 16.2 ± 1.2 respectively). The results of study suggested that the Tibetan may be more advantageous than Han in oxygen transport.

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